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Anaerobic digestion of crop and waste biomass: Impact of feedstock characteristics on process performance

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Department of Biotechnology

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Abstract

Anaerobic digestion provides an array of positive environmental benefits such as reducing greenhouse gas emissions, replacing mineral fertilizers, producing renewable energy and treating waste. However, pitfalls in anaerobic digestion such as poor methane yields, process instability, process failure and regional shortages of feedstock have limited the full exploitation of the anaerobic digestion process.

The research presented in this thesis deals with the assessment of the possible negative or positive impacts of feedstock characteristics on the efficiency of anaerobic digestion. In addition, it investigates ways of enhancing the methane yield of the feedstock by improving the feedstock characteristics. The feedstocks investigated were various energy crops, food industrial waste and sewage sludge. The improvement methods investigated were ensiling, nutrient supplementation, co-digestion and anaerobic pretreatment.

It was found that ensiling crops results in insignificant losses in energy, total solid and wet weight. In addition, no significant difference was found in methane yields between the ensiled and fresh crop samples. The importance of correcting for losses of volatiles in total solids determination was pointed out and it was shown that failing to do so could be the main reason why many previous publications report increased total solid based methane yields after ensiling. Increased methane yield in silages may therefore be an effect of an analytical error rather than an effect of using ensiling as a pretreatment prior to anaerobic digestion.

Anaerobic digestion of crop biomass is known to be particularly limited by nutrient availability. Direct nutrient supplementation in crop mono- digestion in this research demonstrated an efficient biogas process at the shorter hydraulic retention times commonly applied in co-digestion of crop biomass and manure. The high degradation efficiency was evidenced by high methane yields, comparable to maximum expected yields generated under controlled conditions, and low volatile fatty acids accumulation. As a result of nutrient addition, the digestate could comply with certification standards for bio-fertilizer. Also, viscosity problems commonly reported for crop mono-digestion were not observed in this study, which could be another effect of nutrient addition.

Co-digesting of waste biomass and crop biomass led to significant improvement in methane yield per ton of feedstock and carbon to nitrogen ratio as compared to digestion of only the waste biomass. Biogas production from crops in combination with waste biomass also eliminated the need for addition of micronutrients normally required in crop mono-digestion. Co-digestion was also presented as a means of feedstock supplementation to curb feedstock shortages in waste-based anaerobic digestion processes. In addition, inhibitors in anaerobic digestion such as free ammonia and light metal ions were diluted, a condition which can lead to an overall viable biogas process

Anaerobic pre-treatment led to the solubilisation of particulate organic matter in sewage sludge. This solubilisation could have led to the improved methane yield, methane production rate and reduction in volatile solids.

Applying different feedstock improvement solutions to the various feedstocks investigated, i.e. nutrient addition, co-digestion and pretreatment, were demonstrated as effective means of enhancing the methane yield of the feedstock thereby improving the overall anaerobic digestion process.

Key words

Anaerobic digestion, Biofertilizer, Renewable energy, sustainable operation

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PREFACE

The research presented in this PhD thesis was conducted at the Department of Biotechnology, Lund University, under the supervision of Dr Lovisa Björnsson (main supervisor) and Dr Jing Liu (co-supervisor). I greatly appreciate the financial assistance from Eon gas Sverige.

The thesis has two main parts: an introduction, summarising my research, its findings and applications, and a compilation of published papers or papers earmarked for publication. The work described is the result of the collaboration and support of a good many people. In particular, I would like to thank my main supervisor, Lovisa Björnsson for her encouragement, enthusiasm, availability and tireless efforts in making this work a success. The support I received from you was truly first rate. Jing Liu, my co-supervisor, thanks for introducing me into the field of biomethanation or AD and for showing me how to write a scientific article. Marika Murto, thanks for the guidance during the potato waste project.

I would like to thank my colleagues, Emma and Carla, for suggesting improvements in the methane potential test and in the analysis of organics, respectively; and for sharing their great deal of expertise. I would also like to thank Jesper Christensson, Nforngang, J. and Toungwa, F. for their technical assistance at the pilot plant. Thanks to Siv and Frans-Peder for their help with administration and technical issues, respectively. A hearty thanks to all the staff and lecturers at the Department of Biotechnology.

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To you Cameroonians in Lund (CamLund) and Malmö (Camgrass), especially the Kimbungs, the Lekunzes, the Bumus and the Tanahs, you made Sweden (Lund/Malmö) a home away from home. Efetobore Erukporo, you have always been a 'big brother', thanks for all the good counsel.

I would like to extend special thanks to my family, especially my wife Benedicta, my son Darin Achu, uncle Boss, Auntie Boss, uncle Joe, Mr Ngang Cletus, Mr George Atanga, Manka, Chi, Yong, Dominic, Tche etc. who have been extremely patient and have provided tremendous support and love throughout the course of this project. Thanks to you all and many more thanks to the rest of the extended family. Stella Mai, Mrs Nkuo aka Na Jack, Mrs Ataindum aka Na Eric, Hyacintha Wallang aka nawain, the warm fufu-corn and Kati Kati still warms my heart! And you Mr Ngong Kits, Ngong Ludwig, and the Anbainbus, you have been family!

Yotah Joana aka Auntie Joan, you have been a mother, counselor, mentor, provider, etc. I couldn't have asked for more, I appreciate!

Finally, all reverence and praise to Yahweh, the giver of wisdom, knowledge and peace.

Abstract

Anaerobic digestion provides an array of positive environmental benefits such as reducing greenhouse gas emissions, replacing mineral fertilizers, producing renewable energy and treating waste. However, pitfalls in anaerobic digestion such as poor methane yields, process instability, process failure and regional shortages of feedstock have limited the full exploitation of the anaerobic digestion process.

The research presented in this thesis deals with the assessment of the possible negative or positive impacts of feedstock characteristics on the efficiency of anaerobic digestion. In addition, it investigates ways of enhancing the methane yield of the feedstock by improving the feedstock characteristics. The feedstocks investigated were various energy crops, food industrial waste and sewage sludge. The improvement methods investigated were ensiling, nutrient supplementation, co-digestion and anaerobic pretreatment.

It was found that ensiling crops results in insignificant losses in energy, total solid and wet weight. In addition, no significant difference was found in methane yields between the ensiled and fresh crop samples. The importance of correcting for losses of volatiles in total solids determination was pointed out and it was shown that failing to do so could be the main reason why many previous publications report increased total solid based methane yields after ensiling. Increased methane yield in silages may therefore be an effect of an analytical error rather than an effect of using ensiling as a pretreatment prior to anaerobic digestion.

Anaerobic digestion of crop biomass is known to be particularly limited by nutrient availability. Direct nutrient supplementation in crop mono-digestion in this research demonstrated an efficient biogas process at the shorter hydraulic retention times commonly applied in co-digestion of

crop biomass and manure. The high degradation efficiency was evidenced by high methane yields, comparable to maximum expected yields generated under controlled conditions, and low volatile fatty acids accumulation. As a result of nutrient addition, the digestate could comply with certification standards for bio-fertilizer. Also, viscosity problems commonly reported for crop mono-digestion were not observed in this study, which could be another effect of nutrient addition.

Co-digesting of waste biomass and crop biomass led to significant improvement in methane yield per ton of feedstock and carbon to nitrogen ratio as compared to digestion of only the waste biomass. Biogas production from crops in combination with waste biomass also eliminated the need for addition of micronutrients normally required in crop mono-digestion. Co-digestion was also presented as a means of feedstock supplementation to curb feedstock shortages in waste-based anaerobic digestion processes. In addition, inhibitors in anaerobic digestion such as free ammonia and light metal ions were diluted, a condition which can lead to an overall viable biogas process

Anaerobic pre-treatment led to the solubilisation of particulate organic matter in sewage sludge. This solubilisation could have led to the improved methane yield, methane production rate and reduction in volatile solids.

Applying different feedstock improvement solutions to the various feedstocks investigated, i.e. nutrient addition, co-digestion and pretreatment, were demonstrated as effective means of enhancing the methane yield of the feedstock thereby improving the overall anaerobic digestion process.

LIST OF PAPERS

I. Kreuger, E., **Nges, I.A**., Björnsson, L. 2011. Ensiling of crops for biogas production: effects on methane yield and total solids determination. *Biotechnology for Biofuels, 4, 44.*

II. **Nges, I.A**., Björnsson, L. High methane yields and stable operation during anaerobic digestion of nutrient-supplemented energy crop mixtures. *Submitted to Biomass & Bioenergy.*

III. **Nges, I.A**., Björn A., Björnsson, L. 2012. Stable operation during pilot scale anaerobic digestion of nutrient-supplemented maize/beets silage. *Accepted for publication in Bioresource Technology.*

IV. **Nges I.A**., Escobar F., Fu X., Björnsson L. 2012. Benefits of supplementing an industrial waste anaerobic digester with energy crops for increased biogas production. *Waste Management 32:53 - 59.*

V. **Nges I.A**, Mbatia B., and Björnsson L. 2012. Improved utilization of fish waste by anaerobic digestion following omega-3 fatty acids extraction. *Accepted for publication in Journal of Environmental Management.*

VI. **Nges I.A**., Liu J. 2009. Effects of anaerobic pre-treatment on the degradation of dewatered-sewage sludge. *Renewable energy 34: 1795-1800.*

My contribution to the papers

The research presented in this thesis was supervised by Dr Lovisa Björnsson except for the research presented in paper VI, where supervision was done by Dr Jing Liu.

Paper I: I performed the biochemical methane potential test, performed the ensiling experiment together with Emma Kreuger, wrote part of the manuscript, read and commented on the paper.

Paper II: I performed the entire set of experiments, coordinated the writing and wrote the major part of the manuscript.

Paper III: I performed most of the experiments, coordinated the writing and wrote the major part of the manuscript.

Paper IV: I performed the biochemical methane potential test, participated in the full-scale data collection, coordinated the writing and wrote the major part of the manuscript.

Paper V: I planned the experiments, performed the major part of the experiments, coordinated the writing and wrote the major part of the manuscript.

Paper VI: I participated in planning the experiments, performed the experiments, coordinated the writing and wrote the major part of the manuscript.

Abbreviations and symbols

AD	Anaerobic digestion
BMP	Biochemical methane potential
CSTR	Continuous stirred tank reactor
EPS	Extra-polymeric substances
FA	Free ammonia
ha	Hectare, 10 000 m^2
HRT	Hydraulic retention time
kWh	Kilowatt hour
LCFA	Long chain fatty acids
OLR	Organic loading rate
SAO	Syntrophic acetate oxidizing bacteria
SRT	Solid retention time
TS	Total solids
Ton	1000 kg
TWh	Terawatt hour
VFA	Volatile fatty acids
VS	Volatile solids

Contents

Popular science summary

In an ever energy hungry world and given the concerns about global warming, depleting reserves of fossil fuels and growing fuel prices, there is an urgent need for alternative renewable energy sources. Biogas production through anaerobic digestion in sync with other bio energy production technologies could replace or partially replace fossil fuels and hence curb greenhouse gas emissions. The digestate from the process can also replace mineral fertilizer leading to an overall sustainable operation. However, the biogas process has suffered a great many setbacks due to problems such as shortage and unavailability of feedstock, poor methane production, process imbalances and even process failure. Most successfully operated processes are thus usually maintained at very long hydraulic retention times (long digestion times) and low organic loading rates. There is therefore a need for improvement and expansion of the anaerobic digestion process.

This thesis is a summary of six papers (I-VI) and represents my research in the field of biomethanation (biogas production) aiming at improving the efficiency of the biogas process through feedstock optimization. Feedstock is food for the microorganisms in the biogas process. The type, composition as well as the presence or absence of vital macro and micronutrients will influence the outcome of the process. The research presented in this thesis deals with the assessment of the possible negative or positive impacts of the characteristics of the feedstock on the efficiency of anaerobic digestion. In addition, it investigates ways of enhancing the methane yield of the feedstock by improving the feedstock characteristics. The feedstocks investigated were various energy crops, food industrial waste and sewage sludge. The improvement methods investigated were ensiling, nutrient supplementation, co-digestion and anaerobic pretreatment.

Contrary to reports in previously published literature, ensiling as a pre-treatment did not improve the methane yield of crop biomass (Paper I). The reported increased methane yields in literature were suggested to be due to the presence of volatile compounds in silage which could have given analytical error. However, our findings indicated that ensiling could enhance the stability of an anaerobic digestion process, as was evidenced by little or no foaming in a silage fed process (Paper III) as opposed to fresh crop fed process, where foaming was rampant (Paper II). In a related pre-treatment study, i.e. anaerobic pre-treatment of sewage sludge prior to anaerobic digestion (Paper VI), solubilisation of particulate organic matter was observed. This could have led to an increase in methane yield and reduction in volatile solids. However, the solubilisation reported here was partly as a result of fermentation, a condition that often leads to the production of volatile compounds. The presence of these volatile compounds can lead to the same analytical error as was observed in the study presented in Paper I. It is therefore very important to thoroughly characterise ensiled (or 'pre-fermented') biomass so as to achieve better quantification of methane yields and of other total solids weighted parameters such as organic loading rate and reduction in volatile solids.

For energy crops, we were able to demonstrate high methane yields comparable to maximum expected yields and process stability as evidenced by low VFAs accumulation in mono-digestion when both macro and micronutrients were supplemented in the process (Papers II and III). In the nutrient supplemented processes, it was possible to apply high organic loading rates at short hydraulic retention times, thereby increasing the treatment capacity through efficient feedstock utilization (Papers II and III). The nutrient addition was also balanced to make the digestate comply with certification limits for heavy metals content in bio-fertilizer for farmland application (Paper III).

For the processes based on food industrial waste, co-digestion especially led to an improvement in methane yield per weight of feedstock and to a stable process through balancing the carbon to nitrogen ratio and diluting toxicants (Papers IV and V). It was also suggested that co-digestion of waste and crop biomass may eliminate the need for the micronutrient supplementation (Paper IV) that was applied in the studies presented in Papers II and III.

In conclusion, this thesis shows that the performance and conversion efficiency of the biogas process can be improved by improving the characteristics of the feedstock. This is relevant for utilizing the limited available biomass in the most efficient manner.

1. INTRODUCTION AND AIM OF STUDY

Anaerobic digestion (AD) of biomass to produce biogas has gained increasing recognition over the years chiefly because of its positive energy balance, the fact that it works as a waste treatment method and that a recycle of nutrients to agricultural land can be created. AD is a biotechnological process that takes place spontaneously in nature in places where there is total or partial absence of oxygen. Such places include *inter alia* marshes, paddy fields, rubbish dumps, digestive tracks of ruminants and the guts of insects such as termites (Garcia et al., 2000).

Biogas has been defined as gaseous or liquid fuel produced from biomass with an energy content originating from methane (Energigas Sverige, 2011). Digestion gas, landfill gas, liquid biogas (LBG) and bio-methane are synonyms of biogas (Energigas Sverige, 2011). Biogas production through AD or biomethanation is a mature technology as evidenced by the increasing number of biogas plants in both developed and developing countries. For example over 6000 biogas plants are in operation in Germany (Kusch et al., 2012). In addition, biogas plants exist both in small domestic scale as in developing countries such as India and China or in larger community scale as in Denmark, Sweden and Germany (Sims et al., 2008). Sims et al. (2008) also reported that 64 TWh per year of energy in the form of biogas was produced in the EU in 2007.

The drive for biogas production as a renewable fuel is also politically motivated. The European Commission's directive on renewable energy has placed a target to be achieved by each member state by 2020, i.e. 20% of energy from renewable sources in energy consumption and a minimum target of 10% for renewable fuel in domestic transport (European Commission, 2009). Sweden has a national goal of reaching

50% of the energy consumption through renewable energy sources by 2020 and reached 47% as of 2009. However, in the transport sector the share of renewables was only 5.7% (Swedish Energy Agency, 2011). The renewable energy used in the transport sector in Sweden is dominated by bio-ethanol and biodiesel, but also include electricity from renewable sources, and biogas (Swedish Energy Agency, 2011).

The advantage of biogas compared to other renewable transportation fuels such as ethanol and biodiesel is the possibility to derive this fuel from a broad variety of substrates or feedstocks. Biogas production can be considered a low-cost technology because of the ability of the microbial consortia involved in the process to degrade a wide range of (low-cost) feedstocks (Bruni, 2010). Feedstock is the 'food' for the microorganisms in the biogas production process; feedstock properties influence not only process efficiency and stability but also the quality of the digestate or liquid effluent in terms of nutrients and contaminants (Schnürer and Jarvis, 2010; Weiland, 2010). Feedstock for biogas production can be anything from residual products such as manure to energy crops that require extensive production input and the use of agricultural land. Some important waste based feedstocks include food industrial waste (including fish sludge) and sewage sludge as exemplified later in this thesis.

Despite the advantages of the AD process, the technology has suffered drawbacks in areas such as low methane yields, incomplete bioconversion, and process instability. Increasing cost of feedstock and operation of digesters below maximum capacity is also occurring as a result of regional shortages of feedstock (Asam et al., 2011). These drawbacks have prevented the full development, smooth operation and use of the AD technology worldwide (AEBIOM, 2009; Ward et al., 2008). Therefore, there is need and room for improvement of the AD process.

There are many ways through which AD can be optimised. In a 2004 review, Yadvika et al. (2004) reported the use of additives, varying process parameters such as temperature and pH, pre-treatment etc. as potential areas for improving AD. AD has also been reported to be enhanced by directly adding desired microbes into the anaerobic digester (Cirne et al., 2006; Mohan et al., 2005; Nielsen et al., 2007b). In a more recent study, Bruni (2010) summarised topics of AD improvement potential into three main groups: monitoring and control, smart reactor design and increasing the methane yield of the feedstock. Also, in a review about optimization of AD, Ward et al. (2008) referred to improving the methane production potential of the feedstock as one of the optimization techniques but concluded that improved monitoring and control was the most important optimisation technique. Optimisation of AD, however, is much more than optimising the technological know-how. In depth knowledge about biotechnical issues such as the nutritional needs of the microorganisms, the maintenance of a healthy microbial mix in the digester, aspects of microbial inhibition, the balance between fermentation and methanogenesis and the feedstock biodegradability are all important for a well-functioning bioprocess. The above-mentioned issues are all directly influenced by the characteristics and quality of the feedstock used.

This thesis presents my research which aims at assessing the possible negative or positive impacts of feedstock characteristics on the efficiency of AD, and investigating ways of enhancing the methane yield of the feedstock by improving the feedstock characteristics. The six papers discussed in this thesis deal with different features of energy crop biomass (Papers I-III) and waste biomass (Papers IV-VI) as AD feedstock. Figure 1 summarises the feedstocks and investigated methods described in the papers.

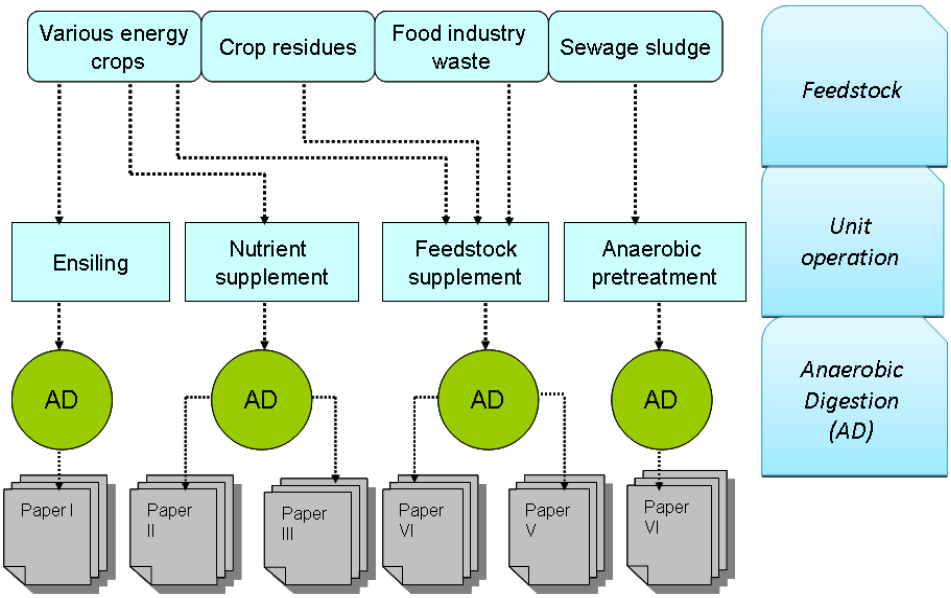

Figure 1. Summary of research and papers presented in the thesis

The goals of the studies performed were different depending on the feedstock investigated. To summarise, the goals were to: (a) study feedstock characteristics such as total solids (TS), volatile solids (VS) and volatile compounds of fresh and ensiled crop biomass and their effects on methane yield determination (Paper I), (b) improve the nutritional balance in AD by direct nutrient supplementation (Papers II and III) or addition of a co-substrate (Papers IV and V) and (c) to investigate whether anaerobic pre-treatment rendered the feedstock more bio-available to the microorganisms (papers VI).

This thesis is divided into six sections; Section 1 is the introduction, Section 2 describes the biogas process and the factors affecting the process, in Section 3, the feedstocks used in this research and their

characteristics are discussed while the research and outcomes of the work investigated are presented in Section 4. The applications of the AD process are discussed in Section 5 and Section 6 presents concluding remarks and future perspectives.

2. THE BIOGAS PROCESS

There are four basic steps involved in AD or the biogas process as outlined in figure 2. They are hydrolysis, acidogenesis, acetogenesis and methanogenesis. Microorganisms involved in the first and second steps are closely linked to each other as are those in the third and fourth steps, thus making it possible to divide the AD process into two phases (Weiland, 2010). A balanced process is one in which the rate of microbial activity is equal in both phases.

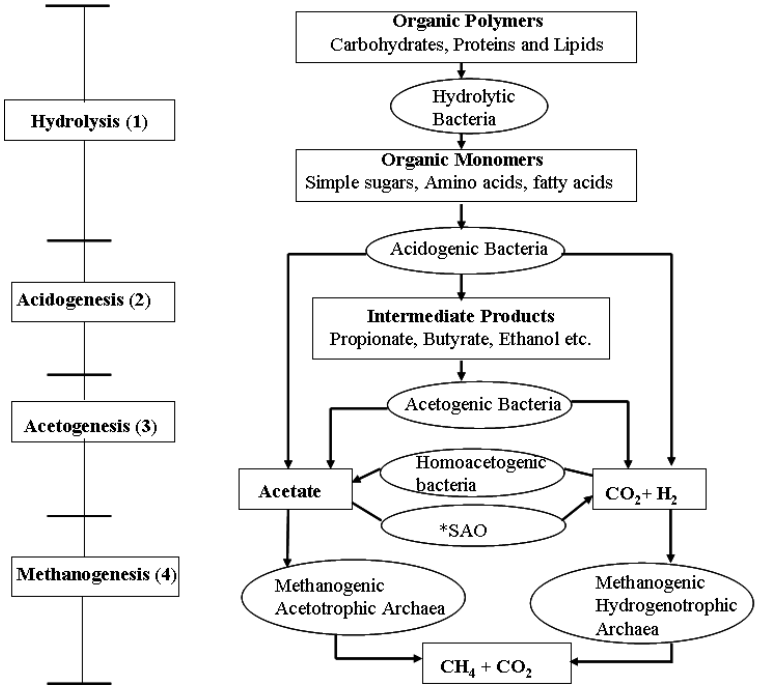

Figure 2. The main pathways in the anaerobic digestion process, modified from Björnsson (2000), Schnürer and Nordberg Å. (2008) and Qu et al. (2009). *SAO refers to Syntrophic acetate oxidizing bacteria.

2.1 First phase

Hydrolysis is often the first step and the rate limiting in AD of particulate organic matter. Polymeric materials such as carbohydrates, proteins and lipids are hydrolysed into smaller, water soluble compounds such as sugars, amino acids, and long chain fatty acids (LCFA) by enzymes produced by the microorganisms (Eastman and Ferguson, 1981). Such microorganisms can either be obligate or facultative anaerobes. The facultative anaerobes play a vital role in this first step as they consume the majority of the oxygen introduced during the feeding of the reactor or digester (Björnsson, 2000). During acidogenesis, hydrolysis products are further broken down by a variety of obligate and facultative fermentative microorganisms to produce organic acids such as acetic acid, propionic acid, butyric acid (VFAs), lactic acid, alcohols, hydrogen and carbon dioxide (CO_2) (Kalyuzhnyi et al., 2000). This step is usually the fastest step in a balanced anaerobic process. The accumulation of lactate, ethanol, propionate, butyrate, and higher VFAs called electron sink or intermediate products is the bacterial response to increased hydrogen concentration in the system (Schink, 1997).

2.2 Second phase

Microorganisms in the second phase are all strict anaerobes. Acetogenic bacteria convert the electron sinks to acetate, CO_2, and hydrogen. This conversion is a vital process because the electron sinks are not utilised by the methanogens and hydrogen accumulation may inhibit the functioning of acetogenic bacteria (Weiland, 2010). Low hydrogen partial pressure (10^{-4} and10^{-6} atm) is required for the acetogenic reaction to proceed (McCarty and Smith, 1986). This low pressure is made

possible by a syntrophic (symbiotic) relationship between the acetogens and the hydrogenotrophic methanogens (Garcia et al., 2000). Methanogens *(Archaea)* utilise acetate, hydrogen and CO_2, and to a lesser extent methanol, methylamines and formate, to form methane and CO_2.

Two major groups of methanogens are known; acetotrophic and hydrogenotrophic, where about 2/3 of the methane is produced by the former. Schnurer and Nordberg, (2008) suggested that in AD processes with high concentrations of free ammonia (FA), acetotrophic methanogens are inhibited, and instead acetate is split into CO_2 and hydrogen by a group of microorganisms called syntrophic acetate oxidizing bacteria (SAO). Hydrogenotrophic methanogens, presumably less sensitive to FA, reduce CO_2 to methane (Schnürer and Nordberg Å., 2008; Schnurer et al., 1999). This tandem pathway of SAO and hydrogenotrophic methanogenesis can produce up to 9/10 of the methane under thermophilic conditions in the presence of high concentrations of FA (Qu et al., 2009).

Accumulation of intermediates or electron sinks can occur when the methanogens are inhibited, e.g. by high concentrations of FA (Lebuhn et al., 2008), deficiency of vital nutrients (Takashima et al., 2011) or process overload (Nielsen et al., 2007a). Propionic acid has been suggested as a good indicator of process imbalance (Nielsen et al., 2007a). It also inhibits anaerobic microorganisms where concentrations of above 1 g/L have been reported to severely inhibit AD (Lebuhn et al., 2008). Other authors have reported butyric acid as the most inhibiting VFA (Ahring et al., 1995) and the ratio of propionic acid to acetic acid has also been suggested as a tool to evaluate process stability (Hill et al., 1987). Due to the complexity of AD, no single parameter may be sufficient to reliably judge the stability of AD. VFA to alkalinity ratio has also been used to evaluate process stability (Papers III, IV, and VII) (Bouallagui et al., 2009; Lebuhn et al., 2008). This could be a better tool

to judge process stability than VFAs alone because it involves the interaction of VFAs, alkalinity and pH.

2.3 Factors influencing the biogas process

The biogas process is influenced by ambient factors, which might slow or stall the process if they are not within a certain range (Angelidaki et al., 2003; Weiland, 2010). Some of the factors observed within the framework of the present study include pH, solid/ hydraulic retention times (SRT/HRT), organic loading rate (OLR) and the presence and availability of key macro and micronutrients.

AD takes place within a pH range of from 6.5 to 8; otherwise, the AD process is inhibited above or below this range (Weiland, 2010). (The optimal range lies between 7 and 8). The pH in AD is usually maintained by the liquid alkalinity, where feedstock characteristics directly influence the alkalinity through the formation of degradation products such as ammonium, bicarbonate, sulfides and phosphates. Ammonium has a large influence on pH and alkalinity, hence large amounts of protein rich biomass are associated with high alkalinity (Paper IV) (Gerardi, 2003).

SRT and HRT, i.e. the mean residence times for solids and liquids, respectively, in a digester, are the same for a suspended-growth AD process such as the single stage continuous stirred tank reactor (CSTR) used in the present study (Gerardi, 2003). All microbial degradation steps in the AD process (Figure 2) take place in the same environment, which might not be favourable for all microbial groups, especially at short HRT and high OLR. However, a nutrient supplemented single stage CSTR treating a mixture of easily hydrolysable and fibrous crops have been reported to function as good as a process where the microbial

groups are separated into the two phases e.g. the leach bed cum upflow anaerobic sludge blanket (Fu et al., 2010).

OLR, i.e. the amount of biomass fed per unit reactor volume per day ($kg/m^3 \cdot d$), is usually based on TS or VS. However, it was found that when OLR is based on TS or VS, the actual load in the digester can be misjudged for 'pre-fermented' feedstock such as ensiled crop biomass (Papers I and III) or feedstock that has undergone a pre-treatment where fermentation was involved, see section 4.1.1. In CSTRs, typical OLR ranges from 1 to 5 kg $VS/m^3 \cdot d$ (Tchobanoglous et al., 2003), but CSTRs are usually operated at the lower range to avoid process instability (Björnsson et al., 2000).

HRT and OLR are mostly used in the design and operation of AD processes. One method shown to be useful for assessing digester performance was evaluation of the maximum OLR that a process can withstand without decreased degradation efficiency (Papers II and III). Processes fed with easily degradable feedstock such as many food industrial waste types are known to be operated at short HRT (15-30 days) while processes fed with feedstocks such as energy crops are operated at HRT of over 100 days (Braun, 2007; FNR, 2010; Weiland, 2010). As described later in section 4.1.2, energy crops can be digested at short HRT with proper nutrient supplementation (Papers II and III).

To continue to reproduce and maintain growth, microbes require sources of energy/carbon (substrate) and nutrients. Unperturbed biogas production requires both macro and micronutrients in sufficient amounts (Gerardi, 2003; Tchobanoglous et al., 2003) (Papers II, III and IV). Excess amounts of some nutrients may however also become inhibitory to the AD process (Paper V) (Chen et al., 2008). A key parameter in the AD process is the carbon to nitrogen (C/N) ratio, which should lie within 15 to 30 for proper functioning AD (Papers II, III & IV) (Schnürer and Jarvis, 2010).

The performance of the biogas process is also influenced by the temperature. As reviewed by Davidsson (2007), AD is usually operated within two distinct temperature ranges, with one optimum at 35 °C (mesophilic) and the other optimum at 55 °C (thermophilic). The research presented in this thesis was performed under mesophilic conditions. Though thermophilic digestion may provide some advantages over mesophilic digestion such as improved reaction rate and pathogen reduction, microorganisms in mesophilic digestion have less demand on nutrients (Takashima et al., 2011) and mesophilic digestion can perform as wells as thermophilic digestion (Nges and Liu, 2010).

3. FEEDSTOCK QUALITY FOR BIOGAS PRODUCTION

In this study, both crop biomass and waste biomass were used as feedstock for biogas production. Feedstock characteristics influence not only process efficiency and stability but may also add value to the overall process by the production of a digestate which can be quality as a bio-fertilizer (Schnürer and Jarvis, 2010; Weiland, 2010). Also, it has been reported that the conversion efficiency of feedstock in the AD process can range from degradation times of near infinity for hard to hydrolyse biomass such as lignin to very short degradation times for low molecular weight compounds such as sugars, alcohols and VFAs (AEBIOM, 2009). In this section, some feedstock characteristics that might lead to poor conversion efficiency and stability are discussed.

3.1 Crop biomass

The crop biomasses experimentally investigated in this thesis were whole crop maize (*Zea mays*), whole crop triticale *(*X *Triticosecale* 'Talus'), hemp (*Cannabis sativa*), sugar beets *(Beta vulgaris)* and the stems/leaves of Jerusalem artichoke *(Helianthus tuberosus)*. The crops were grown in Southern Sweden, fertilized with effluent from a biogas plant and are reputed to have high biomass yield per hectare (ha) (Amon et al., 2007b; Kreuger et al., 2011). The actual harvest times were recommendations from previous studies (Amon et al., 2007a; Amon et al., 2007b; Kreuger et al., 2011b; Tottman, 1987).

Energy crops are increasingly being used as feedstock for biogas production due to increased interest in AD and shortages in waste based feedstock supply for biogas plants (Lindorfer et al., 2007; Sims et al., 2008). It has been estimated that by 2020, 15% of the arable land in Europe will be dedicated to bio-fuel production (Scholz et al., 2010) and

since energy production from crop biomass places a high demand on land, crop digestion processes with high efficiency and performance are a must. Energy crops commonly used as feedstock for biogas production are those with high biomass yield per ha, high biodegradability (high content of easily degradable carbohydrates and proteins), and low fibre content. They should also be easy to cultivate (tolerate pests, weeds, etc.) and to integrate into current crop rotations (Heiermann et al., 2009; Weiland, 2006).

3.1.1 Methane yield from crop biomass

To evaluate the biodegradability of crops in AD, the methane yield is a valuable indicator since high biodegradability is reflected in high specific methane yields as given per TS or VS. These methane yields are often derived from laboratory scale biochemical methane potential (BMP) trials, which should give the maximum expected methane yield from a sample. However, there exists in the literature a large variation in the given methane yields from crop biomass; in fact the same crop variety or species may be reported to have significantly different methane yields (Amon et al., 2007b; Bruni et al., 2010). For example, methane yields from sugar beets have been reported to range from values as low as 230 to values as high as 507 m^3/ton VS (Björnsson et al., 2001; Bohn et al., 2007; Oechsner et al., 2003). In a review of methane yields from different crop biomass, by Braun (2007) reported methane yields of sunflower to range from 177 to 400 m^3/ton VS, alfalfa from 324 to 500 m^3/ton VS, nettle from 120 to 420 m^3/ton VS etc. Different results should be expected if the methane yield is given as the result of a BMP trial or from a continuous full scale processes. But even results from BMP trials vary greatly. These variations can be explained by factors such as differences in crop harvesting time, in the parts of the crop used (e.g. kernel or whole crop plant), in duration or in other conditions of the BMP test, in handling/ pre-treatment, or in

whether the crop sample was fresh or ensiled and there is of course the human factor. Ensiling is a factor that probably brings about much of the variation in methane yield of crop biomass but that is often overlooked.

Ensiling is a common method of preserving crop biomass and has also been described as a pretreatment step prior to AD that could improve methane yields (Pakarinen et al., 2011; Vervaeren et al., 2010). The ensiling process mimics partly the first phase in the AD (Figure 2), where lactic acid bacteria (present in or added to the crops) ferments the easy fermentable carbohydrates to produce lactic acid, alcohols and VFAs (fermentation products). These products are high methane yielding compounds (Weissbach, 2009). They are also volatile, and are often partially or totally lost during oven drying (Paper I) (Porter and Murray, 2001).

Oven drying alone is the most common method for determination of TS or VS (when followed by burning) in ensiled crop samples, but will give underestimated values if the sample contains volatiles that evaporate during drying. Therefore, it can be problematic and even misleading to use TS and VS as the bases for reporting methane yields of ensiled crop biomass or biomass containing VFAs and alcohols since underestimation of TS or VS will give an overestimation of TS or VS based methane yield. Pakarinen et al. (2008) reported methane yields of ensiled grass to range from 360 to 510 m^3/ton VS. Fresh maize has been reported to show a methane yield of 326 m^3/ton VS (Amon et al., 2007b) while ensiled maize showed a methane yield of 418 m^3/ton VS (Vervaeren et al., 2010). Most of these published methane yields are based on uncorrected VS. It is therefore imperative to carefully analyse and correct for volatile compounds for reliable quantification of VS based methane yields, OLR, VS reduction, etc. in AD processes.

3.1.2 Poor nutrient content in crop biomass

A major drawback in using crop biomass as feedstock for biogas production is poor nutrient concentrations, i.e., concentrations lower than the minimum requirements for AD processes (Hinken et al., 2008; Lebuhn et al., 2008; Scherer et al., 2009; Takashima et al., 2011). Both macro and micronutrients are vital for the AD process and their deficiencies have been shown to cause problems in the microbial degradation chain (Pobeheim et al., 2010; Scherer et al., 2009). As a consequence of such nutrient deficiencies, it is common to apply very long HRT in crop mono-digestion. HRTs of up to 228 days have been reported from German and Austrian CSTR-type AD plants (Braun, 2007; Braun et., 2009; FNR, 2010). Poor conversion efficiency and even process failure have also been reported as due to deficiency in nutrient supply to AD processes (Weiland, 2010). These nutrients are integral parts (coenzymes or cofactors) of enzymes involved in the biochemistry of methane production (Demirel and Scherer, 2011; Gustavsson, 2011; Plugge et al., 2009; Takashima et al., 2011). Nutrient deficiency in crop digestion can be partly compensated for by co-digestion of crops with manure (Cavinato et al., 2010; Comino et al., 2010). However, there is regional scarcity of manure (Lebuhn et al., 2008), hence many crop based biogas plants are operated without or with little manure addition (FNR, 2010; Scherer et al., 2009; Weiland, 2010).

The nutrient content in the crop biomass not only influences the performance and stability of the AD process (Hinken et al., 2008; Lebuhn et al., 2008; Scherer et al., 2009) but also the digestate quality as a bio-fertilizer. Therefore, for a feasible crop based AD process and also for the digestate to comply with certification for use as bio-fertilizer for farmland application, both macro and micronutrient supplementation in crop mono-digestion is the approach investigated in this study.

3.2 Waste biomass

Sewage sludge and food industrial wastes of different types are the waste biomass investigated as feedstock in the present study. The sewage sludge was composed of primary, secondary and tertiary sludge from municipal waste water treatment that was dewatered with the aid of flocculent based on polyacrylamide. Food industrial waste was composed mainly of pig manure, slaughterhouse waste, vegetable processing waste and poultry waste. Another investigated food waste sample was fish sludge generated from fish waste as a residue after oil and protein hydrolysate extraction (Mbatia et al., 2010). Slurries such as food industrial waste and sewage sludge have high water content (low TS). Generally, slurries with high water content (TS less 10%) are not economically viable as feedstock for biogas production because of low methane production per unit feedstock and because of the requirement for reactors of large volumes (Asam et al., 2011). The sewage used in this study was thickened in order to partly overcome this limitation, i.e. to reduce the digester volume (Davidsson, 2007).

3.2.1 Food industrial waste

Food industrial waste biomass is often an attractive feedstock for the AD process due to its high content of lipids and proteins, which gives theoretically high methane content and methane yields (Cirne et al., 2007; Braun, 2007; Pereira et al., 2005). Nitrogenous waste such as proteins can also generate high buffering in AD through the production of ammonium (Björnsson, 2000), which can enhance process stability. However, degradation products such as FA and LCFAs can also be present at levels which are inhibitory to the methane forming microbes (Chen et al., 2008; Björnsson, 2000; Chen et al., 2008; Pereira et al., 2005; Rinzema et al., 1994; Schnurer and Nordberg, 2008). This can sometimes lead to process instability and even process failure

(Luostarinen et al., 2009; Schnurer and Nordberg, 2008). It is common for VFAs to accumulate in a FA inhibited AD process. The toxicity of FA and VFAs are pH and temperature dependent, as has been discussed and reviewed in the literature (Björnsson, 2000; Chen et al., 2008; Schnürer and Nordberg Å., 2008). Poor process performance has been reported by Hansen et al. (1998) for cases when there was high concentration of both FA and VFAs in an AD process, termed inhibited steady state. The fish sludge investigated in this study was very high in lipids, which has been known to cause LCFA inhibition when used as feedstock for AD. In addition, this feedstock had high content of light metals such as sodium, potassium, calcium, etc, which are toxic or inhibitory to the AD process at certain concentrations as reviewed by Chen et al. (2008).

3.2.2 Sewage sludge

Sewage sludge is a product of municipal wastewater treatment. As of 2005, there were about 40300 wastewater treatment plants in the European union producing over 9 million tons of TS (Warwrzynczyk, 2007). More than half of the total biogas produced in Sweden in 2009 originated from sewage sludge (Energigas Sverige, 2011). For this feedstock, hydrolysis is usually rate limiting in the microbial degradation chain due to its particulate nature (bacterial cells, lignocellulosic matter such as hygienic paper, various polymeric compounds and floc-organised structure). However, it is rich in both macro and micronutrients (Warwrzynczyk, 2007). Although inherent hydrolytic enzymes in sewage sludge may break down these complex polymers, the degradation is hardly ever effective. This has led to long HRT and low gas yields in sewage sludge AD (Carrère et al., 2010; Climent et al., 2007; Nges and Liu, 2010; Valo et al., 2004).

4. IMPROVING FEEDSTOCK CHARACTERISTICS FOR EFFICIENT BIOGAS PRODUCTION

This thesis deals with the enhancement of the AD process through improving feedstock characteristics. Improvement in feedstock characteristics was evaluated in experimental trials by assessing process stability and the methane yield responses to changes in HRT and OLR. The experimental work was carried out in both batch BMP assays in laboratory scale, and in CSTR experiments (laboratory and pilot-scale).

4.1 Improving feedstock characteristics of crop biomass

In this section, three interrelated topics are discussed: the impact of ensiling crop biomass on methane yield and on TS/VS determination as well as the effect of nutrient supplementation on crop biomass mono-digestion. The effect of nutrient addition on the process effluent (the digestate) as bio-fertilizer for farmland application is also reported.

4.1.1 Methane yield of ensiled crop biomass

The ensiling of four crop biomass samples (maize, beets, beet tops and hemp) and subsequent methane production were investigated in the study presented in Paper I. Many scientific studies report high methane yield for silages (Amon et al., 2007a; Bohn et al., 2007; Herrmann et al., 2011; Lehtomäki and Björnsson, 2006; Pakarinen et al., 2011). These high methane yields have been suggested to be due to partial fiber degradation or increases in concentration of organic acids and alcohol during ensiling. However, most of the reported improvements in methane yield are based on uncorrected TS and VS, which could be the reason for the apparent methane yield increase. The effects of uncorrected TS and VS are illustrated in figure 1, Paper I, where methane yields before and after ensiling for four crops are presented.

The wet weight (ww) and TS based methane yields did not differ significantly before and after ensiling when the TS was corrected for volatiles. When the TS was not corrected for the loss of volatile compounds, however, the ensiled beet roots showed an apparent 51% increase in methane yield (Paper I). It is therefore important to correct for volatile loss during drying in TS determination so as not to overestimate the methane yields of silages. There were also insignificant changes in ww and TS during ensiling (Table 2, Paper I) and loss of energy containing gases such H_2 and CH_4 were also minimal. These losses in volatiles and the correction of TS are important observations, as many of the reported methane yields for energy crop silage in the literature are likely overestimated (e.g. FNR, 2010; Koch et al., 2009; Pakarinen et al., 2008; Vervaeren et al., 2010). In the studies on ensiled energy crops presented in this thesis, care was taken to evaluate TS-based methane yields with correction for volatile compounds in the silage (Papers I and III).

It should be mentioned that ensiling product such as ethanol has a higher theoretical methane yield than does glucose or acetic and lactic acids, 731 m^3/ton VS, as compared to 374 m^3/ton VS, respectively (Braun, 2007; Weissbach, 2009). It has been shown also that well-preserved silage has increasing concentrations of ethanol as a function of the age of the silage (Weissbach, 2009). Based on these reports, it is plausible that ensiling in some cases in fact enhances methane yields of crop biomass.

As a result of the production and loss of CO_2 during ensiling, it was shown in the present study that a silage fed process was better in terms of stability than a fresh crop fed process. In the silage fed process, little or no foaming occurred in the reactor (Paper III) as compared to when fresh biomass was added. In the latter case, foaming was frequent, which thereby jeopardized process stability (Paper II). It should be noted that though it was possible to improve this feedstock properties by

ensiling, the ensiling process is usually marred by losses of high quality compounds such as ethanol. Losses in energy terms of from 8 to 20% have been reported during ensiling of crop biomass for biogas production (Weiland, 2010). Mass losses of up 15% during ensiling have also been reported by Braun (2007). Consequently, for ensiling to be beneficial to the biogas process, mass and energy losses must be minimized.

4.1.2 Nutrient supplementation in crop mono-digestion

It has been reported previously that what had appeared to be unexplainable reasons for process instability or process failure in AD were often found to be caused by deficiencies in nutrients, and it has been suggested that nutrient supplementation could be a means to alleviate process instability and improve methane production (Demirel and Scherer, 2011; Takashima et al., 2011). Micronutrients are sometimes added in energy crop digestion to maintain microbial activity, growth and multiplication, and could also offer the possibility of applying higher OLR and attaining more efficient feedstock bioconversion (Fermoso et al., 2009; Gustavsson et al., 2011). Both macro and micronutrients are vital for the continuous functioning of the biogas process (Scherer et al., 2009; Takashima et al., 2011); therefore, it would be grossly inadequate to only add micronutrients or only macronutrients to an AD process (Gerardi, 2003). It should be borne in mind, on the other hand, that excessive amounts of some nutrients may become inhibitory to the anaerobic microorganisms (Demirel and Scherer, 2011) though bioavailability is often governed by a range of factors, e.g. complexing, (co-) precipitation, pH, temperature, adsorption, etc (Gustavsson, 2011).

In this thesis, nutrient supplementation during long-term AD of fresh and ensiled energy crop mixtures in laboratory and pilot scale CSTRs

were studied (Papers II and III, respectively). The aim was to investigate whether nutrient addition would improve the feedstock characteristics in ways that could be reflected in high methane yields and stable process operation. Table 1 shows the nutrients and their concentrations added in these studies, and the wide range of micronutrient concentrations recommended in the literature as reviewed by Schattauer et al. (2011).

Table 1. Nutrients added in the present study and their role in anaerobic digestion, their concentrations, and typical recommended concentration ranges reported in the literature (Schattauer et al., 2011).

Nutrients	Added concentration (mg/L)	Ranges of recommended concentration (mg/L)	Physiological function/role in methanogesis
Nitrogen (N)	1600-2500	/	Cell component/buffering
Phosphorous (P)	260-420	/	Proteins component, synthesis of ATP
Sulfur (S)	308-385	0.3-13000	CODH / Protein component
Iron (Fe)	30-46	>0.3-4800	Formly-MF-dehydrogenase, CODH, dehydrogenase
Nickel (Ni)	0.5	0.005-5	CH_3-reductase factor F_{430} complex, CH_3-CoM transferase
Cobalt (Co)	1.9-2.2	<0.001-10	CODH, methyltransferase
Molybdenum (Mo)	1.4-1.7	<0.001-50	Formate dehydrogenase

ATP= adenosine triphosphate, CODH= carbon-monoxide dehydrogenase, MF= methanofuran, CoM= coenzyme M, CH_3= methyl

Apart from the enzymatic functions of the micronutrients listed in Table 1, there also exist non enzymatic functions such as electron transfer in microbial respiration processes where metal ions e.g. Fe (III) or Ni (II) act as electron acceptors (Zandvoort et al., 2006). Metal ions or micronutrients in this case are analogous to O_2 in aerobic respiration. Usually, the oxidation of organic matter is coupled to metal reduction and this can be energy yielding to the anaerobic bacteria e.g. Fe (III) and Mn (IV) reducing bacteria (Lovley, 1993).

The species of macro and micronutrients added in the processes was based on previously reported stabilizing and stimulatory effects (Gerardi, 2003; Hinken et al., 2008; Lebuhn et al., 2008; Plugge et al., 2009; Scherer et al., 2009). Methanogens are known to be stimulated by various micronutrients but Fe, Ni and Co are required by all methanogens (Zandvoort et al., 2006). The amounts added were based on recommendations from other studies (Schattauer et al., 2011; Scherer et al., 2009; Takashima et al., 2011) and were in some cases many times higher than the minimal stimulatory concentrations previously reviewed by Scherer et al. (2009) and Takashima et al. (2011).

The fresh crop mixtures used in the laboratory scale CSTR experiments were whole sugar beets (B), beets and maize (BM) and beets, maize and triticale (BMT). The HRT was varied from 30 to 40 days while the TS-based OLR was gradually increased from 1.5 to 5.5 $kg/m^3 \cdot d$. Nutrient addition in the processes was decreased with increasing OLR as the nutrient contribution from the crops was increased.

In the pilot scale trials (Paper III), maize and beets silage was used as feedstock. The trials were run at a constant HRT of 50 days while the OLR was gradually increased from 1.7 to 4.2 $kg/m^3 \cdot d$ based on corrected TS (TS_{corr}) (Paper I). Nutrient supplementation was maintained at the same level throughout the experiment and care was

taken to restrict the amount of nickel (Ni) since it is a heavy metal that is undesirable in digestate used as bio-fertilizer.

Laboratory scale results demonstrated that the processes could be operated at relatively short HRTs while maintaining high methane yields comparable to maximum expected methane yields achieved in BMP assays (Figure 1, Paper II). Stable processes were achieved up to OLR of 4.5 kg/m^3·d, as evidenced by low VFAs concentrations, neutral pH and low VFAs to alkalinity ratio (Figure 2, Paper II). However, for the triticale fed process (BMT), increasing concentrations of VFAs, especially of propionic acid, were observed at OLR 4.5 kg/m^3·d (Figure 2, Paper II). This led to a decrease in substrate conversion efficiency as evidenced by the poor methane yield. In fact the BMT process crashed when the OLR was increased from 4.5 to 5.5 kg/m^3·d. Meanwhile processes B and BM were operated at the final OLR of 5.5 kg/m^3·d, though with resulting decreased methane yields.

Though micronutrients have been reported to stimulate the AD process and particularly aid in the degradation of VFAs such as propionic acid (Demirel and Scherer, 2011; Takashima et al., 2011), VFAs accumulated in the BMT process despite the addition of these micronutrients. The cause of propionic acid accumulation has been inferred in some studies to be the result of FA inhibition (Lebuhn et al., 2008; Takashima et al., 2011). In this study, this was not found to be the case, as crop mixtures B and BM with similar FA concentrations did not exhibit the same tendency. It should be pointed out that there was no VFA accumulation in the sugar beet process (B), while some accumulation (though not severe) was observed in the maize fed process (BM). Also, the same amount of nutrients was added to all the processes.

The above experimental observations led to the hypothesis that VFAs accumulation and eventual process failure in the BMT process was the

result of some intrinsic characteristics of triticale and maize. Cereals such as triticale and maize are rich in phytic acid, a compound known to strongly bind to, or chelate, metal ions such as Fe, Ni, Co, etc. (Pejin et al., 2009), and this may have reduced the bioavailable concentration of essential nutrients to very low amounts (Zandvoort et al., 2006). This phenomenon is well known in animal husbandry, where phytase is usually added to improve mineral uptake for e.g. pigs when fed with cereals (Adeola, 1995). We have however not found any studies on this in relation to biogas production. Phytic acid has also been reported to inhibit methanogenesis (Deublein and Steinhauser, 2010). It should be mentioned though that the micronutrients could have been precipitated as sulfides, which are known to have very low solubility products, especially when the sulfide ages with time from amorphous to crystalline forms (Gustavsson, 2011; Zandvoort et al., 2006). However, metal solubility increases with decreasing pH (Zandvoort et al., 2006). At the same time, solubility and bioavailability do not appear to be absolutely connected since micronutrients such as Ni in the form of sulfides have been reported to be taken up by microorganisms in the AD process (Gustavsson, 2011).

Results from the pilot scale study demonstrated high methane yields comparable to maximum expected yields at constant HRT of 50 days up to an OLR of 3.4 $kg/m^3 \cdot d$ (Figure 2, paper III). Stable operation was also achieved, which was evidenced by low VFA concentration, low VS in the effluent, and low residual methane production (Figure 3 and Table 3, Paper III). At the final OLR of 4.1 $kg/m^3 \cdot d$, increasing concentration of VFAs (up to 1.2 g/L total VFAs), especially butyric acid, were noted. This was accompanied by increased residual methane production, higher VS, increasing extra polymeric substance (EPS) content in the effluent and decrease in the methane content in the biogas. All the above effects meant that microbes could not efficiently degrade the feedstock cumulating to a decrease in methane yield. It should be noted however that though there was a significant decrease in

methane yield at OLR of 4.1 kg/m^3·d, the pH in the digester remained neutral due to the high buffering conferred to the process by nutrient (N) supplementation.

Unlike other studies on crop mono-digestion (FNR, 2010), no viscosity problems were observed in this study. A hypothesis is that this is an additional benefit of nutrient addition. The rheological characteristics of the effluent were studied at OLR 3.4 and 4.1 kg/m^3·d. Though EPS increased significantly from OLR of 3.4 to 4.1 kg/m^3·d, no problems with viscosity were seen (Paper III). EPS secretion has been reported to be promoted as a result of deficiencies in nutrients such as S, P and potassium (K) (Sutherland, 2010). Increased concentrations of EPS may be the reason for the viscosity problems observed in the crop based German CSTRs (FNR, 2010). The effluent viscosity curves and flow diagrams from the pilot scale trials in this study showed that the effluent was of a viscoplastic (pseudo-Newtonian) and thixotrophic nature. The thixotrophic nature meant that some energy or effort (yield stress) is needed for the fluid to start flowing. When however this force is stopped or when stirring is stopped, the effluent will regain its original properties. Hence, intermittent stirring cannot be recommended for a process like the one investigated in Paper III.

4.1.3 Digestate quality

As already stated, the digestate produced in the biogas process can be used as a bio-fertilizer. This feature offers a sustainable recycling of nutrients, improves soil carbon content, and is also the application that is generally most cost efficient (Ahlgren and Börjesson, 2011; Lantz et al., 2009). Most Swedish food producers or farmer organizations approve of applying digestate as bio-fertilizer in food/feed crop production, but require that the digestate should be certified according to the voluntary certification scheme SPCR 120 (Berglund, 2010; SP,

2010). This certification scheme has guideline values for heavy metals such as cadmium (Cd), lead (Pb), chromium (Cr), copper (Cu), mercury (Hg), Ni and zinc (Zn) (Table 2). The amount of bio-fertilizer that can be added is limited by the P or N content and soil class, which in southern Sweden means an average annual addition of bio-fertilizer to reach 22 kg P/ha over 5 years, or 150 kg/ha for easily available N (SJV, 2010).

Table 2. Heavy metals content for farmland applications of digestate from crop digestion without and with the experimentally studied nutrient additions (Papers II and III).

	Laboratory scale trials						Pilot scale trials		Guideline/limit values
	B		*BM*		*BMT*				
	Without (-) or with (+) nutrient additions								
	-	+	-	+	-	+	-	+	
t/ha	63	35	56	34	59	35	105	32	
	kg/ha								
N*	174	153	169	152	155	147	223	156	150
P	22	22	22	22	22	22	22	22	22
K	99	55	83	50	86	50	96	30	
	g/ha								
Cd	**7.4**	**4.1**	**5.4**	**3.3**	**2.8**	**1.6**	**2.6**	0.8	0.75
Pb	6.2	3.5	5.8	3.5	4.7	2.7	**30**	9	25
Cr	9.8	5.5	9.3	5.7	7.7	4.5	34	10	40
Cu	72	41	61	37	48	28	210	65	300
Hg	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.2	1.0
Ni	7.7	21.8	7.6	21.3	4.9	20.7	24	20	25
Zn	266	150	246	149	193	114	333	102	600

For the digestates, N is given as total N. The limit values are however based on easy available N.

Based on P-addition of 22 kg/ha, the field application amounts of digestate were calculated. From this, the applied amounts of the desired nutrients and the unwanted heavy metals were calculated. The

calculation has been made for all the crops used in the long-term trials in this study (Table 2).

As a result of the low content of P in relation to naturally occurring heavy metals in the crops none of the digestates from the crops/crop mixtures investigated in the laboratory scale trials in this study complied with certification guidelines (Paper II). In the pilot scale trial, this aspect was then taken into account and as a result of the adjusted P addition in the AD process the effluent complied with SPCR certification (Paper III). Without nutrient supplementation, 105 ton/ha of effluent would have to be spread to reach 22 kg P/ha. This would have given too high additions per ha of Pb and Cd. When nutrients were added, all heavy metal amounts were within or below guideline values. In laboratory scale, Cd amounts were reduced by nutrient addition but did not fall within the guideline values.

The addition of nutrients in the pilot scale trials (Paper III) was based on experience from prior laboratory studies (Paper II), and the P-addition in the pilot scale trial was adjusted so that the Cd-limit in the bio-fertilizer would not be exceeded. This approach to determining optimum amounts of nutrients could be a way of enabling certification of the digestate. It should also be noted that the heavy metals occur naturally in the crops in the study region, and that levels of Cd are especially high in sugar beet tops. The common practice is that the beet tops are left on the field after harvest, and then no restrictions on Cd amounts are applied.

The rheological character of the effluent may also influence the applicability of the effluent as a good bio-fertilizer. The viscosplactic behaviour of the effluent (Paper III) meant that it can easily seep into the soil, thus reducing the risk of nitrogen losses by ammonia volatilisation. Ammonia evaporation from surfaces applied with biogas effluent has been shown to be lower than from surfaces applied with pig

slurry (Birkmose, 2007). It has also been reported that humic substances present in the digestate can facilitate nutrient uptake from the soil (Atiyeh et al., 2002), leading to higher nutrient concentration in the crop. Addition of macronutrients therefore not only contributes to the stability of the biogas process and gives efficient substrate degradation, but also makes the digestate more attractive as a bio-fertilizer.

To conclude, nutrient addition at the levels investigated in these studies will increase the overall operational cost. This could however be justified by the high methane yields, stability and the overall high productivity of the process (more feedstock being converted to methane at short HRT and high OLR). Also the digestate utilization as a bio-fertilizer could provide potential economic benefits and could offset the cost of the nutrients added to the process. It should be noted that the amounts of nutrients added in this study were not optimized, but were set at quite high levels. It may be possible to reduce the amount of nutrients (Table 1) and still have an equally stable process, especially as there are concerns over global shortages of natural resources such Ni and Co (Demirel and Scherer, 2011).

4.2 Improving feedstock characteristics of waste biomass

The impact of co-digesting waste and crop biomass, i.e. co-digestion of lipid/protein rich biomass with carbohydrate-rich biomass, as well as the effects of anaerobic pre-treatment of sewage sludge prior to AD are reported in this section.

4.2.1 Co-digestion and feedstock supplementation

For AD to be economically viable, a continuous supply of feedstock is required, but this procedure is not always possible due to increasing

competition for feedstock (Lindorfer et al., 2007). Consequently, there is a need for appropriate feedstock supplementation in order to deal with fluctuations in feedstock supplies (Lindorfer et al., 2007). Co-digestion technology can also be used to abate the inhibitory effects that can occur with lipid and protein rich feedstock and improve nutrient imbalance (Luostarinen et al., 2009; Murto et al., 2004).

Co-digestion of lipid/protein rich waste biomass and crop biomass was investigated in laboratory batch BMP trials and continuous CSTR processes with a view to upgrade to a full or commercial scale plant (Paper IV). The motivation was to respond to the recurrent shortage of food industrial waste as feedstock in full scale processes. In the full scale process studied in Paper IV, the FA concentration was deemed to be at the inhibitory threshold. Therefore, mixing of energy crops and the industrial waste had a dual function of nutrient balancing and feedstock supplementation.
Results from chemical analyses of the crop samples and industrial waste demonstrated that the crops were poor in both macro and micronutrients. On the other hand, the industrial waste was rich in these nutrients (Table 1, Paper IV). Co-digesting energy crops and industrial waste can therefore also be a means of achieving efficient digestion of energy crops without nutrient addition (Demirel and Scherer, 2011).

The results of co-digestion showed improvement in C/N ratio and reduced FA concentration, conditions which could lead to a better process performance. There was a significant (32 %) improvement in methane yield per ton of feedstock as compared to the watery food industrial waste slurry alone (TS of about 10%) (Figure 2, Paper IV). TS in the co-digestion feedstock suggested in the present study was 16%. Thickening of feedstock for biogas production has been reported to be desirable, as it reduces the volumetric load to the digester (Schnürer and Jarvis, 2010). It should also be mentioned that crop addition to food

industrial waste can cause problems with pumps clogging and flotation of biomass if the process is designed for low TS feedstock.

Co-digestion of fish sludge and the above the ground part of Jerusalem artichokes was also investigated (Paper V). In a previous study, essential oils in the form of omega-3 fatty acids and fish protein hydrolysate were extracted from the fish waste generating the fish sludge (Mbatia et al., 2010). High methane yields were achieved both for the fish sludge and the fish waste, i.e. 742 m^3/ton VS and 828 m^3/ton VS, respectively (Figure 2, Paper V). The difference in yields was a result of the extracted oils or lipids, which have higher theoretical methane yields than proteins (Moller et al., 2004). However, chemical analysis showed high concentrations of light metals (Table 1, Paper V), which, together with high concentrations of lipids and protein degradation products (LCFAs and FA), could inhibit methanogenic microorganisms.
The feasibility of co-digesting the fish sludge with a carbohydrate-rich residue from crop production was thus demonstrated, and a full-scale process outlined for converting fish waste to multiple useful products. Co-digestion, exemplified in this study by a residue from crop production, could mitigate the potential inhibitory effect of FA (Table 2, Paper V), light metals and LCFAs, as these inhibitors are degraded or diluted to acceptable levels. Through AD, fish sludge can be converted from an odorous residue to a renewable energy carrier and a high-quality bio-fertilizer (Table 3, Paper V).

4.2.2 Anaerobic pre-treatment

The efficiency of the AD of particulate biomass such as sewage sludge may be improved by incorporating a pre-treatment step that will enhance its hydrolysis, thereby producing easily digestible low molecular weight compounds (Bruni, 2010; Carrère et al., 2010;

Climent et al., 2007; Davidsson, 2007). Several pre-treatment methods have been reported in the literature with the ultimate goal of solubilising the particulate biomass, making it easily accessible to the anaerobic microorganisms. These methods include chemo-thermal, mechanical, ultrasonic and ozone treatments (Carrère et al., 2010; Climent et al., 2007). Chemical and high temperature thermal treatments have been reported to be the best options, but they are based on strong acidic or basic conditions in combination with high temperatures and pressures (Chu et al., 2002; Nah et al., 2000; Valo et al., 2004). In addition, the above pre-treatment options have been shown to be either too expensive or to result in the formation of toxic refractory compounds leading to poor methane yields (Carrère et al., 2010).

The effects of low temperature anaerobic pre-treatment on sludge solubilisation in order to improve its biodegradability were investigated on dewatered sewage sludge (Paper VI). Pre-treatment was carried out at 25 ^{o} C, 37 ^{o}C, 50 ^{o}C and 70 ^{o}C for 12 h, 1 day, 2 days and 3 days. Two control samples were included, one untreated and the other autoclaved (121 ^{o}C for 20 minutes). The pretreated and control samples were evaluated for methane production in BMP assays. Best results were achieved with the anaerobic pre-treatment at 50 ^{o}C for 3 days, which led to a 23 % COD solubilisation (Table 2, Paper VI) and a subsequent 11% improvement in methane yield (Figure 3, Paper VI). The solubilisation represented the transfer of organic matter in the particulate form to the soluble fraction (Bougrier et al., 2006). The solubilisation was considered to be both thermal (carried out at 50 ^{o}C) and biological (caused by hydrolytic enzymes inherent in sludge microbes). Also, though the overall digestion time was not affected by pre-treatment, 90% of the methane was produced within the first 12 days for the samples pre-treated at 50 ^{o}C, as compared to over 2 weeks for the other treated samples (Figure 1, Paper VI). Generally, VS reduction was also improved from an average of 42% to 51%, while the methane content was on an average 69% for the treated samples, 7%

higher than the untreated samples (Table 3, Paper VI). In a similar low temperature pre-treatment prior to the AD study, a 50% improvement in methane yield was noted after 70 ^{o}C pre-treatment of secondary sludge (Climent et al., 2007).

In this study, losses in volatile compounds during oven drying were not accounted for, which is stated as important for proper evaluation of samples containing volatile compounds (Paper I). It is possible that there was vaporization of volatile compounds produced during pre-treatment during TS or VS determination. This could have influenced the outcome of this study and other studies in the literature where fermentation is part of the pre-treatment. Care should thus be taken when reporting increments in TS or VS based methane yields or VS-reduction after such pre-treatment, as the yields may be overestimated as a consequence of losses in volatile compounds (Paper I).

5. APPLICATION OF BIOGAS AND CO-PRODUCTS

Methane is used in today's society in everything from vehicle fuel, to heat and steam production, electricity generation, combined heat and power (CHP) generation, production of chemicals, etc. (Energigas Sverige, 2011). Figure 3 depicts a CSTR based biogas plant where the feedstock is handled as slurry. The feedstock undergoes and pre-sanitation step at 70 °C for 1 h.

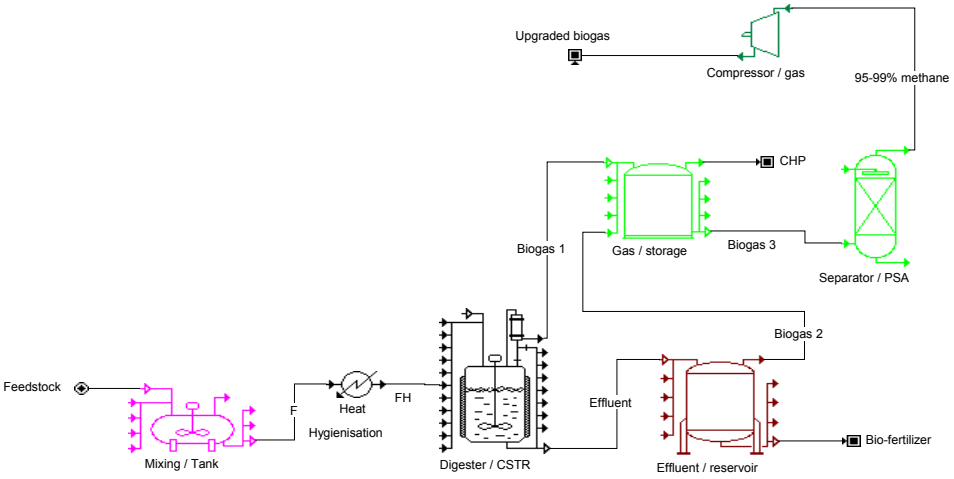

Figure 3. Schematic presentation of the entire biogas process: F denotes homogenised feed, FH hygienised feed, CSTR continuous stirred tank reactor, PSA pressure swing adsorption and CHP combined heat and power

The biogas is used for CHP or upgraded by any of various upgrading techniques, e.g. pressure swing absorption (PSA), which enables use of methane as vehicle fuel. The digestate can also be used as a bio-fertilizer. Below, some methane applications together with other

products from the AD process are presented, which together can be operated sustainably forming a close circuit (Braun, 2007).

5.1 Renewable energy and reduction of greenhouse gas emissions

Biogas in sync with other bio-fuels can partially offset the use of fossil derived fuels. Apart from heating, cooking, lighting, etc., biogas is currently being upgraded and then used as vehicle fuel. Biogas production in the EU is predicted to reach 1930 TWh by 2020 (AEBIOM, 2009). In Sweden, 1.4 TWh was produced in 230 biogas plants in 2010; 49% was used for heating, 36% upgraded and used as vehicle fuel, 5% for electricity while the rest was flared. Biogas as vehicle fuel has earlier been reported to be resource efficient and the most environmentally sound fuel as compared to bioethanol and rapeseed methyl-esters or bio-diesel (Börjesson and Mattiasson, 2008). By 2008, about 17000 biogas driven cars were in operation in Sweden with over 120 filling stations (AEBIOM, 2009).

As of 2011, over 44 million households used biogas made in farm or household-scale anaerobic digesters for lighting and cooking in developing countries (REN21, 2011). In Cameroon, for example, manure fed farm-scale biogas digesters are being operated in rural areas. This has led to an improved standard of living through job creation and also combated deforestation (Ngalame, 2012). However, the introduction of biogas technology for cooking and lighting in developing countries has been limited to animal owners, as manure is seen as the sole feedstock for biogas production (REN21, 2011).

Replacing fossil fuels with bio-fuels such as biogas will greatly reduce greenhouse gas emissions. The reduction of the greenhouse gases are greater, and the emissions of nitrogen oxides (NOx), hydrocarbons and

particles are less with methane than with the combustion of conventional fuels (Berglund and Börjesson, 2006; Energigas Sverige, 2011).

5.2 Waste treatment and production of bio-fertilizer

Amongst all the bio-energy harnessing technologies, AD is the only process that combines bio-energy harnessing with possible waste treatment and production of bio-fertilizer. As compared to other waste treatment options such as composting, AD has the advantages of high volume reduction, positive energy balance and low biomass generation (Williams, 2005). Macronutrients such as N, P and S are retained in the digestate, making it a valuable bio-fertilizer (Papers III and V). In Sweden there is a positive attitude toward using digestate as bio-fertilizer in the food/feed industry.

It should be stressed here that recycling of plant nutrients such as P is vital since peak phosphorous is eminent and the remaining quality of phosphate rock (the main source of phosphorous) is decreasing while at the same time the price of phosphorous is increasing (Cordell et al., 2009). In addition, if nutrients are not efficiently recycled in agriculture, they may seep into bodies of water such as rivers and lakes, causing eutrophication (Sharpley et al., 2001).

5.3 Application of carbon dioxide

Combustion of biomass or biomass derived energy is considered neutral with respect to CO_2, so-called biogenic CO_2 (Munack and Krahl, 2007). It has been reported that global warming can be reduced in fossil fuel production sites by combined carbon capture and storage (CCS) (Sims et al., 2008). Bio-energy production with carbon capture and storage (BECCS) could be a greenhouse gas mitigation technology which

produces negative carbon emissions by combining biomass use with carbon capture and storage (Obersteiner et al., 2001). Though CCS has gained acceptance as to greenhouse gas reduction, concerns about CO_2 leakages remain. An alternative technology is carbon capture and utilization (CCU) (Yu et al., 2008). CO_2 produced during upgrading of biogas can be used in green houses to improve vegetable (crop) yields. Leiv M, (1987) have earlier reported improved crop production as a result of CO_2 enrichment. CO_2 can also be used for the production of an array of valuable compounds and alternative energy sources, i.e. methanol by CO_2 hydrogenation (Quadrelli et al., 2011).

5.4 The bio-refinery concept

It is economically and environmentally important that all components of a feedstock used in a biochemical process can be fully converted to usable end products. This can be achieved in a bio-refinery. A bio-refinery is analogous to a petroleum refinery which produces multiple fuels and chemicals; it has been defined as a facility that integrates biomass conversion processes and equipment to produce fuels, power, and value-added chemicals from biomass (NREL, 2007). Bio-based industrial products can only compete with petro-chemical based products when biomass resources are processed optimally through bio-refinery systems (Kamm et al., 2006).

The bio-refinery concept was explored in the study presented in Paper V. Fish waste was previously converted to low-volume, high value omega-3 fatty acids and fish protein hydrolysate, while the waste from the fatty acid extraction, called fish sludge, was used to produced a low-value, high volume renewable fuel (biogas) through AD. The effluent from the AD process was qualified as bio-fertilizer because nutrients such as N, P and K were retained in the effluent. Heavy metals were within the acceptable range for certification according to SPCR 120

(SP, 2010) and the fish waste had previously gone through a pre-sanitation step (Mbatia et al., 2010). Despite the seemingly numerous advantages reported for the bio-refinery, it still remains a concept since a full scale bio-refinery has yet to be constructed.

6. CONCLUDING REMARKS

By 2020, 60% of the biogas produced within the EU is predicted to come from crop biomass and the rest from waste biomass (AEBIOM, 2009). However, the characteristics of crop biomass such as poor nutrient content have led to poor process performance (long HRTs and low OLR) in crop based AD. For some types of waste biomass, degradation products such FA and LCFAs are known to be inhibitory to anaerobic microorganisms. AD of solid biomass is also limited by hydrolysis in the microbial degradation chain. Thus, both crop and waste biomass suffer from drawbacks as AD feedstocks. An efficient bioprocess can therefore be achieved by improving the characteristics of the feedstock by balancing nutrients, diluting inhibitors and improving bioavailability, thus enhancing biodegradability.

This thesis opines that performance and conversion efficiency in the biogas process can be improved by improving the characteristics of the feedstock. Also fermentative pre-treatment/storage processes such as ensiling may impact positively on biogas process performance or methane yields as well as on stability.

Nutrient supplementation, co-digestion and pre-treatment were employed to enhance the biodegradability of feedstock, i.e. to improve the methane yield. The different methods reported in this thesis showed improvement in methane production as a consequence of balance in the microbial degradation chain, improved process stability or enhanced feedstock bioavailability.

The results of the present study demonstrated that ensiling did not improve the methane yield of crop biomass when the TS/VS were corrected for volatile compounds that are lost during oven drying (Paper I). It is therefore very important to thoroughly characterise ensiled (or

'pre-fermented') biomass when it is used as feedstock for biogas production.

High methane yields, comparable to maximum expected yields, and process stability as evidenced by low VFAs accumulation were achieved in the mono-digestion of nutrient supplemented crop biomass. As a consequence of nutrient addition, it was possible to apply high OLRs at short HRTs, thereby increasing the treatment capacity through efficient feedstock utilization (Paper II and III). The nutrient addition was also balanced to make the digestate comply with certification limits for heavy metal content in bio-fertilizer for farmland application (Paper III).

Co-digestion of waste biomass with crop biomass led to an improvement in methane yield per weight of feedstock and a stable process through balancing the C/N ratio and diluting toxicants (Paper IV and V). Through co-digestion of waste biomass and crop biomass, it was also possible to eliminate the need for micronutrient supplementation commonly applied in crop biomass anaerobic digestion (Paper IV).

Solubilisation of particulate biomass and improved biodegradability were noted with pre-treatment (Paper VI), where anaerobic pre-treatment of sewage sludge could have led to an increment in methane yield and VS reduction.

For an even better process performance, it is also possible for two or more of the reported feedstock improvement methods to be carried out in tandem. This was the case with energy crop mono-digestion, where ensiling followed by nutrient supplementation (Paper III) gave a more stable process as evidenced by little or no foaming and higher methane content in the biogas as compared to nutrient supplementation without prior ensiling (Paper II).

The work reported in this thesis therefore contributes to the canon of knowledge in the field of AD and it highlights the need to increase the methane yield of the feedstock as a means of improving the efficiency of the biogas process.

Some issues raised during this study indicate the need for further considerations. Further studies are needed in areas such as factors affecting the bioavailability of metal ions (micronutrients) in the biogas process. It was postulated in this study that compounds such as phytic acid can chelate or form complexes with micronutrients, rendering those nutrients less bio-available (Paper II). Further studies on the effect of phytic acid on the biogas process are needed to verify this hypothesis.

The effects of microbial products such as EPS on the viscosity of biogas digestate also need further studies. In this study, it was hypothesised that increased EPS concentrations in crop mono-digestion are due to deficiencies in nutrients such as S, P and K. High concentrations of EPS could be the cause of viscosity problems seen in nutrient deficient crop digestion processes. Additional studies are needed to elucidate the correlation between EPS, nutrient content, and viscosity in the biogas process (Paper III).

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Paper I

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Ensiling of crops for biogas production: effects on methane yield and total solids determination

Emma Kreuger*, Ivo Achu Nges and Lovisa Björnsson

Abstract

Background: Ensiling is a common method of preserving energy crops for anaerobic digestion, and many scientific studies report that ensiling increases the methane yield. In this study, the ensiling process and the methane yields before and after ensiling were studied for four crop materials.

Results: The changes in wet weight and total solids (TS) during ensiling were small and the loss of energy negligible. The methane yields related to wet weight and to volatile solids (VS) were not significantly different before and after ensiling when the VS were corrected for loss of volatile compounds during TS and VS determination. However, when the TS were measured according to standard methods and not corrected for losses of volatile compounds, the TS loss during ensiling was overestimated for maize and sugar beet. The same methodological error leads to overestimation of methane yields; when TS and VS were not corrected the methane yield appeared to be 51% higher for ensiled than fresh sugar beet.

Conclusions: Ensiling did not increase the methane yield of the studied crops. Published methane yields, as well as other information on silage related to uncorrected amounts of TS and VS, should be regarded with caution.

Keywords: biogas, anaerobic digestion, methane potential, biofuel, ethanol, volatile fatty acids, dry matter, total solids, volatile solids, ensiling, silage

Background

Biogas production using energy crops as the main feedstock is attracting increasing attention. Germany is leading the field, with almost 3, 900 biogas plants in operation in 2009, the majority using ensiled crops [1]. Ensiling is a traditional method of preserving animal feed, and can also be used to store crops intended for biogas production [2]. The amounts of total solids (TS) or dry matter (DM) and volatile solids (VS) are often used to characterize the ensiled material added to the biogas process, and to calculate the methane yield from the material. A standard method of determining the TS of biomass is oven drying at 105°C [3,4]. Other oven temperatures, such as 60°C, 85°C or 100°C are also common [3,5]. In this paper total solids (TS) and dry matter (DM) are regarded as being equivalent, and the term used is that used in the publications referred to.

At the beginning of the 20th century it was reported that oven drying gives inaccurate values of the DM when the sample contains volatile compounds. It should therefore not be applied to silage as it contains varying amounts of volatile fatty acids (VFAs), lactic acid, ammonia and alcohols formed during the ensiling process [6,7]. McDonald and Dewar [8] quantified the losses of volatile compounds during oven drying by condensing and analyzing the vapor. A year later, they described a method in which the water content was determined through toluene distillation, with corrections for organic acids, ethanol and ammonia in the distillate [9]. The corrected toluene extraction method was long used as a standard method for determining the DM in silage used for fodder production, but was abandoned due to the harmful nature of toluene. The most common method used today to determine the DM in silage is oven drying, with corrections for the volatilization of VFAs, lactic acid, alcohols and ammonia. The type and amount of volatile compounds lost depends on the drying temperature, and different coefficients are used to adjust the DM for the expected losses of individual

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compounds at certain drying temperatures [5,10]. The adjusted values are referred to as corrected DM or corrected TS.

Although the limitations of using oven drying without correction for volatile compounds have been known for many years in agricultural sciences, the method is still routinely used in research related to methane production through anaerobic digestion. The methane yield from anaerobic digestion is normally expressed per unit of VS. The amount of VS is based on the amount of TS, which is determined according to standard methods by oven drying, without correction for volatile compounds [4]. After oven drying, the dry material is incinerated at 550°C to determine the ash content. The difference between the TS and the ash is defined as the VS. This means that if the TS are underestimated the VS will also be underestimated. If the VS of the silage are underestimated, the loss of VS during ensiling will be overestimated, and the methane yield per unit VS will be overestimated.

VS losses of 18% to 35% due to ensiling have been reported [11]. At the same time, ensiling has been reported to increase the methane yield of the material by 25% to 42% [11,12]. Results such as these may be the result of losses of volatile compounds during VS determination. There are several other recent examples of this, where the methane yields reported from ensiled grass, maize and beet were based on methods of TS or VS determination without correction for the loss of volatile compounds (see, for example, [13-17]). The VS-based methane yields given for ensiled materials may therefore be overestimated. Yields from silage based on uncorrected TS and VS values have been reported in other biofuel fields as well, such as ethanol research [18,19].

Although no biogas-related research has, until very recently [20], made use of the thorough internationally published studies performed on silage for fodder, some authors have considered the fact that volatile compounds may be lost during the determination of TS and VS. It is mentioned in the standard method of the American Public Health Association (APHA) [4] that losses of volatile organic matter from the sample can cause a negative error, but no further comments are made on how this error can be corrected. Angelidaki *et al.* [21] suggest drying at a lower temperature (90°C) after increasing the pH of the sample. However, according to Porter and Murray [5], neither drying at lower temperature nor increasing the pH decreased the volatilization of alcohols. Demirel and Scherer [22] described a method of VS determination applied to beet silage, in which suspended solids and dissolved solids (VFAs, lactic acid and alcohols) were analyzed separately, by drying and gas chromatography, respectively, and then combined to give the total VS. However, dissolved organic compounds other than VFAs, lactic acid and alcohols will not be included. Methods, including volatilization coefficients, have been presented in publications by Weissbach and Strubelt [23-26] and Mukengele and Oechsner [27] in a German journal for agricultural technology. Volatilization coefficients for correcting oven-dry-based DM for ensiled crops are outlined, and the methods described are similar to that presented by Porter and Murray [5]. Unfortunately, these articles will not be found via scientific search engines such as ISI Web of Science, Scifinder and SciVerse ScienceDirect, and the articles refer to methods published in German (see, for example, [28]). Two recent publications [20,29] concerning the influence of ensiling on the methane potential do make use of correction factors [10,28]. However, none of them emphasize the importance of correcting TS and VS, to avoid overestimating methane yields, and both refer to previously published results based on uncorrected TS and VS without comment or concern about the reliability.

Among others, McDonald *et al.* [30] have pointed out that, even when using corrected DM, the change in DM during ensiling does not provide a measure of the change in the energy content of the silage, since the two are not correlated (as can be seen in Table 1). The fermentation of sugar to acetic acid or lactic acid will not influence the potential for methane production (Table 1). Fermentation to ethanol results in the concentration of the energy in the dry matter, and part of the dry matter is lost as carbon dioxide, while most of the energy is retained in the product (Table 1). The stoichiometric methane potential of glucose, acetic acid and lactic acid is 374 l/kg VS and, for the more reduced carbon source ethanol it is 731 l/kg VS. Only in cases of undesirable fermentation, such as butyrate fermentation, is a considerable amount of energy truly lost due to the release of hydrogen (see Table 1). In well preserved silage, the butyrate concentration is low [30].

The purpose of the current study was to examine how ensiling influences the methane potential, the mass and the total solids of crops. Furthermore, we wished to draw attention to the errors that can arise from using uncorrected, oven-dry-based values of TS and VS, and to highlight a previously presented method, for correcting oven-dry-based TS and VS values for losses of volatile fermentation products during oven drying [5]. The method developed for grass silage was tested on four other crop materials. Laboratory-scale ensiling was performed, followed by methane production from ensiled and non-ensiled crops. The losses in wet weight, and the production of methane and hydrogen and total gas volume during ensiling were determined. The content of the dominating volatile organic compounds in silage were measured before and after standard TS

Table 1 Mass and energy recovery for fermentation during ensiling

Type of fermentation	Product	Mass recovery	Energy recovery
Homolactic fermentation	$2C_3H_6O_3$	100%	97%
Acetic acid fermentation	$3C_2H_4O_2$	100%	93%
Heterolactic fermentation	$C_3H_6O_3 + C_2H_6O + CO_2$	76%	97%
Ethanol fermentation	$2C_2H_6O + 2CO_2$	51%	97%
Butyrate fermentation[a]	$C_4H_8O_2 + 2CO_2 + 2H_2$	49%	78%

Mass and energy recovery for some common fermentation pathways during ensiling [30]. The examples are based on glucose as substrate. Gases are regarded as lost. Energy recovery is based on the gross energy value (higher heating value) of the products, excluding the energy in ATP.

[a]Performed by many *Clostridia* species.

determination of the ensiled crops and used to calculate corrected TS and VS contents. The TS and VS contents were corrected in two ways: one using the volatilization coefficients presented by Porter and Murray [5], and the other (for validation) by adding the fraction of volatile compounds lost during drying. The volatilization coefficients from Porter and Murray [5] were used since they are based on silages mainly prepared with bacterial inoculants [5] rather than silages prepared with formic acid [10]. Four crop materials were chosen for the study: maize, which is the dominating crop used for anaerobic digestion in Europe; hemp, which is more fibrous than maize; and sugar beet (beets and beet tops ensiled separately), which contain less fiber and more soluble sugars than maize.

Results and Discussion

Comparison of the changes in wet weight, TS and VS during ensiling based on uncorrected and corrected values

The wet weight was found to decrease during ensiling by about 1% for all materials except beets, for which the decrease was about 4% (Table 2). For sugar beets and maize, the decrease in TS during ensiling was significantly higher than the decrease in wet weight when using the uncorrected TS content, demonstrating the error in the method (rows E and F in Table 2). After correcting the TS contents of the silages the decrease in TS (row K, Table 2) was no longer larger than the decrease in wet weight for any of the materials.

Table 2 Changes in wet weight (WW) and total solids (TS) during ensiling

Row		Percentage of	Maize	Hemp	Beets	Beet tops
A	Ensiling replicates, n		4	2	3	4
B	TS prior to ensiling[a]	Fresh WW	26.8 ± 0.2	31.4 ± 2.1	23.0 ± 0.2	13.2 ± 1.6
C	VS prior to ensiling[a]	Fresh WW	25.0 ± 0.1	28.4 ± 0.4	21.3 ± 0.9	10.6 ± 0.6
D	Uncorrected TS after ensiling[b]	Silage WW	24.5 ± 0.8	29.4 ± 0.4	14.2 ± 0.1	10.4 ± 0.4
E	Weight after ensiling	Fresh WW	99.2 ± 0.0	98.4 ± 0.1	95.6 ± 0.3	99.0 ± 0.5
F	Decrease in TS based on uncorrected TS[c]	Fresh WW	2.5 ± 0.8	2.4 ± 2.1	9.5 ± 0.2	2.9 ± 1.6
G	Maximum CO_2 relased[d]	Fresh WW	0.5	1.5	3.3	0.7
H	TS after ensiling based on CO_2 release[e]	Silage WW	26.5	30.4	20.6	12.6
I	Corrected TS after ensiling according to Porter and Murray[f]	Silage WW	26.4 ± 0.1	30.7 ± 0.5	23.3 ± 1.1	13.1 ± 0.7
J	Corrected TS after ensiling based on measurements[g]	Silage WW	26.5 ± 0.1	30.4 ± 0.5	23.8 ± 1.1	13.6 ± 0.7
K	Decrease in TS, corrected according to Porter and Murray [5][h]	Fresh WW	0.6 ± 0.2	1.2 ± 2.2	0.7 ± 1.0	0.2 ± 1.8

Changes in W and TS during ensiling, expressed as percentage of fresh crop or silage WW (mean ± SD). TS content was determined in duplicate. Decrease in WW and the maximum amount of CO_2 released were determined for the number of ensiling replicates given in row A.

[a]Measured on fresh crops with ensiling solution.

[b]The TS content was analysed for both ensiled crops directly after opening the buckets (the value given here) and after freezing (the value used for correcting TS and VS, since VFAs, lactic acid and alcohols were determined after freezing). No significant difference was seen between the two measurements.

[c]Calculated according to: B - D × (E/100) (letters indicate rows).

[d]Based on the total amount of gas released and the estimated amount of CO_2 in the ensiling buckets minus methane, and hydrogen and the estimated amount of nitrogen gas in the buckets at the start of ensiling.

[e]Calculated according to: (B - G)/(E/100) (letters indicate rows).

[f]TS values in row D plus 37.5% of the lactic acid, 100% of the ethanol and 89.2% of the acetic and butyric acid present in the silage (Table 3), according to Porter and Murray [5].

[g]TS values in row D plus the difference between the contents of lactic acid, ethanol, acetic acid and butyric acid in the ensiled crops before and after TS determination.

[h]Calculated according to: B - I × (E/100) (letters indicate rows).

TS, total solids; VFA, volatile fatty acid; VS, volatile solids; WW, wet weight.

Ethanol and acetic acid were present in all silages (Table 3). Lactic acid was present in all silages except the hemp silage (Table 3). Butyric acid (Table 3) and very small amounts of propionic and succinic acid (less than 0.1% of the wet weight) were detected in hemp silage, but not in the other silages. The pH of the hemp silage was higher than the other silages; namely 4.5, compared with 3.1 for maize, 3.0 for beet tops and 2.9 for beets.

After drying the silages no ethanol could be detected, and lactic, acetic and butyric acid were found at lower concentrations. On average, 100% (± 0%) of the ethanol (n = 8), 53% (± 13%) of the lactic acid (n = 6), 72% (± 0.01) of the butyric acid (n = 2) and 89% (± 17%) of the acetic acid (n = 8) evaporated during TS determination. The average values are not significantly different from those presented by Porter and Murray [5]: 97.5% for ethanol, 37.5% for lactic acid and 89.2% for acetic and butyric acid. However, there is considerable variation in volatilization between the samples as indicated by the SDs, showing that there is room for further improvement of the method. The volatilization coefficients used by Weissbach and Strubelt [25], included a pH dependency for the VFAs, which may further increase the accuracy of the corrected values. The volatilization coefficients presented in that article cannot be compared to those obtained here since they used different drying conditions (initial drying at 60°C, followed by drying at 105°C) from those used in this study (105°C).

Corrected TS contents are presented in rows I and J in Table 2. The values in row I are calculated based on the concentrations in the silages and the volatilization coefficients given by Porter and Murray [5]. The values in row J are based on the experimentally determined volatilization during oven drying, that is, the difference between the content of volatiles before (Table 2) and after (data not shown) TS determination by oven drying. No significant differences were found between the results obtained with the two methods, showing that the volatilization coefficients presented by Porter and Murray [5] give good estimates of the true TS for the silages investigated. Theoretical calculations of the TS contents after ensiling, based on the gas production and weight changes (row H, Table 2), gave values in line with those obtained with corrections for losses of volatiles (rows I and J, Table 2).

Gas production and energy losses during ensiling

The production of energy-containing gases such as hydrogen and methane during ensiling was negligible in all cases: less than 0.1 ml per g VS for all substrates except hemp, which gave less than 2 ml hydrogen per g VS. The energy contained in the hydrogen produced by hemp during ensiling corresponded to about 2 ppm of the energy in the methane produced in the biochemical methane potential (BMP) test. For hemp, beets and beet tops, only hydrogen and no methane was detected; for maize, methane but no hydrogen was detected. The low production of energy-containing gases, together with the low pH in all the silages, except hemp, indicates that the silages were well preserved.

For maize, hemp and beet tops, 67% to 89% of the total gas produced (including carbon dioxide) during ensiling was produced during the first 4 days. The gas produced by beet silage was higher than that produced by the other crops, with high gas production during the first 4 days, and a second gas production peak around days 9 to 13, giving 72% of the total gas production between days 6 and 17. All crops produced less than 6% of the total gas between days 30 and 60. After 60 days, the buckets were moved from storage at room temperature to 4°C. Very little gas was produced after this, less than 1% by all crops except hemp, which produced around 5% of the total gas during this time.

The maximum mass loss due to aerobic degradation resulting from entrapped oxygen at the start of the ensiling process was calculated and found to be negligible, at most 0.025% of the wet weight. The calculation was based on the assumption that the maximum volume of entrapped air was the volume of the bucket minus the volume of the substrate at the start of ensiling (assuming a density of the substrate of 1 kg/l), 21% of the air being oxygen.

BMP tests

The methane potential was determined and is expressed per unit wet weight (Figure 1a) and per unit uncorrected and corrected VS for silages (Figure 1b). When

Table 3 Volatile compounds in ensiled crops

Substrate	n	Lactic acid	Ethanol	Acetic acid	Butyric acid	Total
Maize	2	1.26 ± 0.02	0.21 ± 0.00	0.74 ± 0.04	BD	2.21 ± 0.05
Hemp	2	BD	0.29 ± 0.01	0.94 ± 0.04	0.11 ± 0.01	1.13 ± 0.04
Beets	2	0.91 ± 0.07	4.82 ± 0.86	1.09 ± 0.14	BD	6.82 ± 0.87
Beet tops	2	1.08 ± 0.04	0.53 ± 0.04	0.56 ± 0.00	BD	2.18 ± 0.06

Contents of volatile compounds measured in the ensiled crops, expressed as percentage of wet weight (mean ± SD). Determinations were made in duplicate starting with the steeping step.

BD, below detection limit.

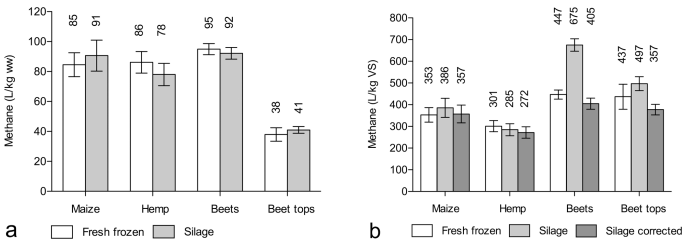

Figure 1 Methane yields of fresh frozen and ensiled crops. Methane yields expressed as **(a)** l per kg wet weight (WW) (left), and **(b)** l per kg volatile solids (VS) (right). The methane yields are given above the bars. Error bars denote 1 SD, n = 3.

expressing the methane yield per unit wet weight (Figure 1a) or per unit VS corrected according to Porter and Murray [5] (Figure 1b) no significant difference was seen between fresh frozen and ensiled material for any of the crops. Neither was there any significant difference between the methane yields from fresh frozen crops and ensiled crops related to the wet weight or VS of the original materials (taking mass losses during ensiling into account).

When relating the methane yield from ensiled material to uncorrected VS, the results are noticeably different. The apparent methane yield from beets was significantly higher (51%) from ensiled material than from fresh frozen material when expressing the yield per unit uncorrected VS (Figure 1b). A significant difference was also seen between the methane yield from silage expressed per unit uncorrected and corrected VS for beets and beet tops (Figure 1b).

Herrmann *et al.* [29] found that the methane yields were significantly higher after ensiling in 44% of the cases investigated, when the methane yields of the silages were related to the corrected VS of the silages, but not when they were related to the original VS. Pakarinen *et al.* [20] found methane yields after ensiling to be everything from unchanged to decreasing or increasing compared to yields from fresh crops. Pakarinen *et al.* [20] did not relate their results to original VS since changes in TS and VS during ensiling were not recorded.

The overestimated methane yield of beet silage and beet top silage in the current study, and the fact that the TS losses appeared higher than the wet weight losses for beets and maize when using uncorrected TS and VS contents, demonstrate that methane yields of silages based on uncorrected TS and VS are unreliable.

Conclusions

Ensiling was not found to increase the methane yield from any of the crop materials investigated in this study. Instead, it was shown that observations such as increased VS-based methane yields or TS losses during ensiling may be artifacts caused by errors in the standard methods commonly used for TS and VS determination. Oven-dry-based TS and VS determination without correction for the loss of volatile compounds is an unsuitable method for all substrates containing noteworthy amounts of volatile compounds. This applies to ensiled energy crops as well as other materials, and is important when using the substrate for anaerobic digestion as well as for other purposes. Caution should therefore be exercised when considering published information about silages, and other materials containing volatile compounds, based on TS and VS. The application of a method developed for grass silage for correcting TS and VS [5], to other ensiled crops, eliminated the significant error of using uncorrected TS and VS. However, the method can be improved further.

Methods

Crops

Hemp (Futura 75), maize (Arabica) and sugar beet (EB 726 (Syngenta, Basel, Switzerland), a non-commercially available cultivar with lower sugar content and higher biomass yield than normal sugar beet) were cultivated in southern Sweden (Lönnstorp, Lomma, 55 40'N 13 6'E). The crops were harvested on the following dates: hemp on 5 September 2007, maize on 29 September 2008, and sugar beet on 21 October 2008. Hemp and sugar beet were harvested manually. Maize was harvested with a maize forager set at a chopping length of 10 mm. The hemp and sugar beet tops (leaves and the neck of the root) were chopped in a garden shredder (AXT 2500 HT, Robert Bosch GmbH, Germany) into pieces about 2 cm long. The sugar beets were cut into 1 cm slices and then into squares measuring 2 to 3 cm. Part of each crop material was ensiled directly and part was frozen for later analysis. The TS and VS contents were determined in fresh crops before ensiling with and without ensiling inoculant, in fresh crops after freezing, and in

ensiled crops before and after freezing. TS corrected for volatile compounds were determined in frozen ensiled crops. (Frozen samples were used since the authors were not aware of the corrected method prior to freezing the silage.)

Ensiling

Ensiling was carried out in 4.8 l plastic buckets with tightly fitting lids, normally used for food storage (NordicPack, Nykvarn, Sweden). Hemp, maize, sugar beets (beets) and sugar beet leaves including the upper part of the roots (beet tops) were ensiled separately, using four replicate buckets for each kind of crop material. A gas collection system was made by connecting Tygon tubing (VWR International, West Chester, PA, USA) to a balloon made of Transfoil El-OPET/PE (Flextrus AB, Lund, Sweden) with a hose connector (Slangservice i Uppsala AB, Uppsala, Sweden) in each lid. Silicone was used to seal the connection between the hose connector and the lid and between the bucket and the lid. The chopped plant material was sprayed with a bacterial ensiling inoculant, Lactisil Stabil (Chr. Hansen A/S, Hørsholm, Denmark). In all, 20 ml was added per kg wet plant material, according to the manufacturer's instructions (1.25 g powder in 5 l tap water). The decrease in weight was recorded by weighing the material in the buckets before and after the ensiling period. The decrease in TS was determined based on the wet weight and TS of the fresh crops with ensiling solution and of ensiled crops.

The buckets were stored at room temperature (23 to 25°C) for 60 days; after which they were stored at 4°C for a minimum of 100 days. The gas volume and the contents of methane and hydrogen were monitored during the entire ensiling period. The results from one bucket of beets and two buckets of hemp were excluded due to gas leakage.

The replicate samples of each crop material were mixed after ensiling before sampling for TS and VS determination, and for BMP tests. The mixed samples were also frozen for later analyses. TS determination and BMP tests were started immediately after sampling to minimize losses due to volatilization during sample handling. Contents of VFAs, lactic acid and ethanol were determined in silage samples that had been frozen, since this part of the study was included later. Prior to analysis, frozen silages were thawed at 8°C in buckets with tightly fitting lids.

BMP tests

BMP tests were performed as reported elsewhere [31], with the modifications described below. Fresh frozen crops, ensiled crops (not frozen) and control samples (described below) were tested in triplicate. The inoculum-to-sample ratio was 2:1 in terms of VS of the fresh frozen crops; silage was added based on the same wet weight as the fresh frozen crops. A total of 300 ml of inoculum was added to each test flask. Inoculum was collected from an anaerobic codigestion plant (Söderåsens Bioenergi, Wrams Gunnarstorp, Sweden). This inoculum is rich in macronutrients and also contains relatively high amounts of trace elements, therefore no nutrients were added. The reaction temperature was set to 38°C. The inoculum was preincubated at 38°C for 5 days prior to the start of the experiment.

The total gas volume and the content of methane [31] were monitored every day for the first week, and then every third or fourth day thereafter, until the end of the experiment. Two sets of controls were included: one set in which only the inoculum was used (to measure the indigenous methane production from the inoculum, which was subtracted from the total methane produced), and a second with microcrystalline cellulose (Avicel PH-101, Sigma-Aldrich, St. Louis, MO, USA) to test the activity of the inoculum. The experiments were terminated after 30 days. The methane yield was related to the wet weight and to the TS and VS of fresh substrate with ensiling inoculant and ensiled substrate. For ensiled substrates the methane yields were also related to the VS content corrected according to Porter and Murray [5]; VS contents determined after freezing were used for this since these were the materials used for determination of the volatile compounds.

Analyses

TS and VS were determined in duplicate or quadruplicate according to standard methods [4], using samples of 13 to 240 g instead of 25 to 50 g. The TS of each substrate were measured several times, for example before and after the addition of ensiling solution, before and after freezing, and so on. In each case, the TS value corresponding to the actual material used was used for calculations. Corrected values of TS and VS were determined similarly to those presented by Porter and Murray [5]. Duplicate samples of 60 g thawed frozen silage (mixture of material from all ensiling replicates) were steeped in 300 g deionized water for 15 to 19 h at 8°C in a 500 ml flask with a lid. For beets and beet tops the material was separated into a solid and a liquid part (6% liquid for beets and 15% for beet tops) before sampling. The pH was measured after steeping and the pH of undiluted silage was calculated. Quadruplicate samples of the same material were analyzed by drying 13 to 41 g wet weight in aluminum crucibles at 100 to 105°C for 20 to 24 h, according to standard methods to determine TS [4]. Two of the quadruplicates of the dried samples were steeped in deionized water in the same proportions as for the wet silage (1:5), and the other two samples were used for VS determination according to standard

methods. Steeping was performed in crucibles covered with several layers of Parafilm. Liquid samples were acidified with H_2SO_4 to a pH of 1 to 3 and filtrated through 0.45 μm polypropylene filters (Chromacol, Welwyn Garden City, UK). The content of C_1-C_6 VFAs (including isoforms of butyric and valeric acid), lactic acid, succinic acid and ethanol were determined using high performance liquid chromatography (HPLC) (Jasco Co., Tokyo, Japan) with an Aminex HPX-87H column (Bio-Rad Laboratories Inc., Hercules, CA, USA) and a refractive index detector (Erc Inc., Huntsville, AL, USA). Sulfuric acid (5 mM) was used as the mobile phase (0.6 ml/min), and the oven temperature was 40°C. The concentration of VFAs, lactic acid and ethanol and were calculated for the wet silage according to Equations 1 and 2:

$$\text{Concentration in wet silage (g/kg)} = (m_1 + m_2 - m_3) \times c_1/m_1 \quad (1)$$

$$\text{Concentration after drying related to wet silage (g/kg)} = c_1 \times D \times m_3/m_1 \quad (2)$$

Where m_1 = original wet weight related to TS added, g; m_2 = water added, g; m_3 = substrate TS added, g; c_1 = concentration of analyzed compound, g/kg; and D = dilution factor = 5.

The TS and VS were corrected in two ways: (1) according to the volatilization coefficients for grass silage dried at 100°C presented by Porter and Murray [5]: lactic acid 0.375, total VFAs 0.892 and ethanol 1.000; and (2) the measured losses of VFAs, ethanol and lactic acid during drying (the difference between Equations 1 and 2) were added to the TS and VS values measured using standard methods.

Gas composition with respect to methane was determined using gas chromatography and a thermal conductivity detector, as described elsewhere [32]. Hydrogen was analyzed in an identical system but with argon as the carrier gas. The gas volume was measured using a graduated 100 ml gas-tight glass syringe (Fortuna, Germany) with a sample lock. Gas volumes are expressed as dry gas at 0°C, assuming a constant pressure of 1 atm.

Statistics

All statistical analyses were performed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test using the statistical software Prism (Prism 5 for Mac OS X, version 5.0b; GraphPad Software Inc., La Jolla, CA, USA). The term 'significant' is only used where a statistical analysis of significance has been performed. The significance level of 5% was used throughout all statistical analyses. Values are given ± 1 SD. The SDs of weight losses during ensiling, of TS and VS determinations, of the concentrations of volatile compounds added to the corrected values of TS and VS and of tests and controls in BMP were combined according to standard statistical rules [33] to provide a SD of the final result. For linear combinations (Equation 3) the SDs were combined according to Equation 4 [33]. For multiplicative expression (Equation 5) the SDs were combined according to Equation 6 [33]:

$$y = k + k_a a + k_b b + k_c c + \ldots \quad (3)$$

$$\sigma_y = \sqrt{((k_a\sigma_a)^2 + (k_b\sigma_b)^2 + (k_c\sigma_c)^2 + \ldots)} \quad (4)$$

$$y = kab/cd \quad (5)$$

$$\sigma_y/y = \sqrt{((\sigma_a/a)^2 + (\sigma_b/b)^2 + (\sigma_c/c)^2 + (\sigma_d/d)^2 + \ldots)} \quad (6)$$

Where σ = standard deviation; a, b, c, d = independent measured quantities; and k = constant.

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Authors' contributions

LB became aware of the methodological problem investigated, and secured financial support for this study. All authors participated in the design of the study, harvesting of the crops and reviewing of the literature. EK set up the ensiling method and performed most of the ensiling experiments, the TS and VS determinations and all analyses of the volatile compounds. IAN participated in the ensiling trials and carried out the BMP tests. EK performed the statistical analysis and prepared the major part of the manuscript. LB and IAN contributed to writing the manuscript, and all authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

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Paper II

High methane yields and stable operation during anaerobic digestion of nutrient-supplemented energy crop mixtures

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Abstract

The feasibility of digesting energy crops supplemented with macro- and micronutrients instead of manure, without the commonly applied long hydraulic retention time (HRT), was investigated in long-term, single-stage continuous stirred tank processes. The crops used were mixtures of sugar beets, maize and whole crop triticale. The organic loading rate (OLR) measured as a total solid (TS) was 1.5 - 5.5 kg $m^{-3}\cdot d^{-1}$ and the HRT from 30 to 40 days. The results showed high methane yields, comparable to those in batch digestion, and high stability. The digestion of beets only was most stable, and showed the highest average methane yield (383 ± 26 m^3 CH_4 kg^{-1} TS) at an OLR of 4.5 kg $m^{-3}\cdot d^{-1}$ and a HRT of 40 days. No significant difference in methane yield was found for all the crop mixtures during stable operation. Nutrient addition therefore showed the same stimulatory and stabilising effects as manure with high methane yields achieved at relatively short HRTs.

Keywords: Anaerobic digestion; Biogas; Energy crops; Macronutrients; Micronutrients

1. Introduction

The use of energy crops alone as the feedstock for biogas production via anaerobic digestion has been found to be prone to instability and even process failure [1-3]. On the other hand, co-digestion of energy crops with manure has been reported to greatly improve the anaerobic digestibility of energy crops [4-5]. This has been attributed to the broad spectrum of nutrients, vitamins and trace metals (micronutrients) found in manure [6]. However, increased interest in anaerobic digestion of energy crops and the scarcity of manure (due to declining stock farming), in Germany, for example, has led to many biogas plants being operated without, or with little, manure [2-3, 7]. Furthermore, the use of manure in biogas production is regulated by EU directives[8], demanding e.g. heat treatment at 70 °C for 1 hour to reduce pathogens when manure from several farms is mixed together. The operational conditions and performance of 45 stirred-tank, mesophilic energy-crop-based biogas plants in Germany have been reported by FNR [7]. The plants using little or no manure (0% to 30% of total feedstock measured as wet weight (ww)) were found to operate at average hydraulic retention times (HRTs) of 170 days, while plants using a manure fraction above 50% had an average HRT of 46 days. Braun [9] also reported a mean value of 140 days HRT in the mono-digestion of energy crops and 50 days when equal amounts of crops and manure are co-digested. It would seem, therefore, that anaerobic digestion of energy crops can not proceed with good biodegradability at short HRTs without manure addition.

The outcome of anaerobic digestion depends on the characteristics of the feedstock [10] as well as the amounts of macro- and micronutrients present. Ensuring adequate availability of nutrients for the microbes is a

problem when single substrates rather than complex mixtures of materials are used in the biogas process[11]. Some authors have concluded that optimal concentrations of phosphorus (P), sulphur (S), potassium (K), magnesium (Mg), iron (Fe), nickel (Ni), molybdenum (Mo), cobalt (Co), tungsten (W), selenium (Se) and zinc (Zn) are needed to afford process stability and high performance in anaerobic digestion[3, 12-13]. Macronutrients such as, nitrogen (N), P and S, and micronutrients such Fe, Ni, Mo, Co, W and Se have been found to play a crucial role in the growth and metabolism of anaerobic microorganisms [14-16]. Macronutrients are known to act as buffering agents [1, 16] while micronutrients have been reported to be abundant in the numerous enzymes (carbon monoxide dehydrogenase, Formyl-methanofuran-hydrogenase, methylreductase, formate-hydrogenase, methyltransferase etc.) involved in the biochemistry of methane formation [13]. Gerardi [12] concluded that both macro- and micronutrients are required for better functioning of the biogas process. Hence it will be inadequate if only macronutrients or only micronutrients are supplied to a biogas process.

It has been concluded in a few studies that the addition of nutrients (in lieu of manure) improved methane production and process stability in the digestion of energy crops. Lei [17] reported that the addition of an adequate amount of P (465 mg L^{-1}) could accelerate the bio-gasification process of rice straw, while [2] reported a boost in methane production as a result of the addition of the same ratio of P and S. The addition of Ni (0.6 mg L^{-1}) and Co (0.1 mg L^{-1}) to the anaerobic digestion of maize silage was found to improve the methane yield by 25% and 10%, respectively[11]. Lebuhn [18] reported a total recovery in an acidified crop-based anaerobic process after the addition of micronutrients.

The above findings are encouraging and warrant further study to investigate the potential of digesting different energy crops with both macro- and micronutrient addition to improve biogas production. Producing biogas from energy crops places demands on arable land as compared to waste-based renewable energy production. Consequently, high process efficiency is particularly important. High conversion efficiency and high biogas yields influence process economy, land use efficiency and the process energy balance [19].

The hypothesis investigated in this study was that energy crops can be digested with good methane yields at relatively short HRTs when selected macro- and micronutrients are added. The aim was not to optimise nutrient addition, but to investigate a set of macro- and micronutrient which have the potential to provide stable operation and high methane yields. Single-stage continuously stirred tank reactors (CSTRs) were used, with HRTs between 30 and 40 days. The organic loading rate (OLR) was increased until process instability /failure occurred. The crops investigated were sugar beet, maize and whole crop triticale, which are commonly used as feedstock in biogas production [7, 20]. In addition to nutrients, the impact of feedstock characteristics on the anaerobic digestion process was also investigated. Three different mixtures of crops were composed and tested in parallel.

(i) Sugar beet roots and beet leaves/tops mixed at the ratio at which they were harvested, i.e. 2:1 based on the weights at harvest i.e. ww (un-dried material).

(ii) Beet roots constituting half of the feedstock, with the addition of equal amounts of beet leaves/tops and maize in based on ww. The reason for studying this mixture was that beet leaves and maize can be stored as silage at a 1:1 ww ratio.

(iii) Whole crop triticale contributing almost half of the feedstock while beet roots, beet leaves/tops and maize made up the rest. The reason for investigating this crop mixture was that an autumn-sown, summer-harvested crop should provide half the methane production, while the other half would be made up of spring-sown, autumn-harvested crops.

2. Materials and Methods

2.1 Feedstock and inoculum

2.1.1 Energy crops

The crops were grown in an energy crop cultivation trial in southern Sweden (Lönnstorp, Lomma, 55 40'N 13 6'E), fertilized with effluent from a biogas plant. Lomma receives an average of 1000 mm of rainfall per year and is 10 m above sea level. The Cultivars chosen were based on high biomass yield rather than quality for food or feed. Sugar beets, *Beta vulgaris* (roots, tops, and leaves), 'Biogas type' (EB 726, Syngenta, Basel Switzerland) a non-commercially available cultivar with high biomass yield was harvested at full maturity (late October). Whole maize plant, *Zea mays* , (Arabica cultivar, stay green type) was harvested at full ripeness (late September) based on the recommendations of [20] for late ripening cultivars. Triticale, '*x Triticosecale talus'*, (a Talus cultivar, fodder type) was harvested as whole crop at the vegetation stage (mid July), where the highest biomass yield per hectare is obtained [20]. Maize and triticale were harvested with a precision chopper set at a chopping length of 10 mm. Beet leaves/tops were chopped in a garden shredder (AXT 2500 HT, Robert Bosch GmbH, Germany) into pieces about 2 cm long. Beet roots were cut into 1 cm slices. The chopped crop samples were weighed and immediately

transported to the experimental site and frozen. Mineral concentrations and total solids (TS) were determined in triplicates for each crop sample prior to freezing. Three different crop mixtures were investigated (based on ww), as follows:

- 67% of beet roots and 33% of beet leaves/tops (denoted mixture B),
- 46% of beet roots, 28% of beet leaves/tops and 26% of maize (denoted mixture BM), and
- 28% of beet roots, 14% of beet leaves/tops, 20% of maize and 38% of triticale (denoted mixture BMT).

The TS were used to calculate the crop: water ratio in feedstock preparation to achieve process operation with a constant HRT. Before use, the crop mixtures were partly defrosted and ground batch-wise in a homogenizer (Grindomix 200, Retsch, Germany) so as to pass through a 4 mm mesh. In CSTRs experiments, the ground crop mixtures were mixed with minerals, and in some cases water, to prepare the feedstock, which was stored for about 5 days in a refrigerator (4 °C) prior to use in the experiments.

2.1.2 Nutrient supplementation

The macronutrients N, P and S were added as salts to the feedstock. N was provided by ammonium chloride (NH_4Cl), ammonium hydrogen carbonate (NH_4HCO_3), ammonium sulphate ($(NH_4)_2SO_4$), which also provided S, and urea ($CO(NH_2)_2$). Initially, the N addition was 50:50 from NH_4Cl and NH_4HCO_3. From day 74, N was added at the following percentages: 48% NH_4Cl, 28% NH_4HCO_3, 14% $(NH_4)_2SO_4$ and 10% $CO(NH_2)_2$. From day 167, half of the urea was replaced by NH_4HCO_3. P

was added in the form of sodium hydrogen phosphate ($NaH_2PO_4 \bullet 2H_2O$). Stock solutions of micronutrients (10 times concentration) were prepared and added to the feedstock to provide Fe, Co, Ni and Mo in the form of iron sulphate ($FeSO_4 \bullet 7H_2O$), cobalt chloride ($CoCl_2 \bullet 6H_2O$), nickel nitrate ($Ni(NO_3)_2$) and ammonium molybdate ($(NH_4)_6Mo_7O_{24} \bullet 4H_2O$), respectively. All chemicals were of reagent grade (Savern & Werner, Sweden).

2.1.3 Inoculum

The inoculum used in the experiments was collected on two separate occasions from a full-scale mesophilic anaerobic digester (Söderåsens Bioenergi, Sweden) treating food industrial waste from different sources, slaughterhouse waste and pig manure. The inoculum had an average pH of 8.2, ammonium nitrogen (NH_4-N) of 3.5 g L^{-1} and a partial alkalinity of 14 g L^{-1}. Other characteristics of the inoculum are presented in Table 1.

2.2 Process operation

2.2.1 Biochemical methane potential test

The different crop mixtures (without nutrient or water addition) were digested in biochemical methane potential (BMP) tests under mesophilic conditions to establish the maximum expected methane yields. The basic experimental set-up was as previously described by [21]. Particularities and exceptions were that all the tests were carried out in triplicate at 37 °C; no pH/nutrient adjustment was made as the inoculum was rich in nutrients, and the inoculum had a high buffering capacity. Two sets of controls were included in each test. First, only the inoculum was used, to measure its intrinsic methane production. A second control containing cellulose

(Avicel PH-101, Sigma-Aldrich, St. Louis, MO, USA) was used to validate the experimental set-up and procedure. Gas composition and total gas volume were monitored every other day during the experiments. The experiments were terminated after 30 days of incubation. The methane produced by the inoculum was subtracted from the results obtained with the test samples.

2.2.2 CSTR experiments

The experimental set-up consisted of six jacketed, 4-L single-stage glass CSTRs. The reactors were initially charged with 3 L inoculum, and this level was maintained throughout the experiment. Impellers (EURO-ST D, Germany) rotating at 1.3 Hz were used to mix the reactor content. The reactors were maintained at 37 °C by circulating hot water inside the reactor water jacket (Newington, USA).

The TS and volatile solids (VS) of the three crop mixtures are given in Table 1. The maximum OLR (base on TS) to be investigated was set to 5.5 kg $m^{-3} \cdot d^{-1}$ where these TS -values would give HRTs for the crop mixtures B, BM and BMT of 36, 35 and 39 days respectively. Based on these values, the HRT was initially set to 30 days, and the OLRs to be investigated to 1.5, 3.0, 4.5 and 5.5 kg $m^{-3} \cdot d^{-1}$. After 143 days of operation, the HRT was reconsidered and set to 40 days. In the preparation of the feedstocks the crops were diluted to give TS concentrations of 4.5%, 9%, 12% and 18% in the feedstock for the combinations of OLR and HRT of 1.5/30, 3.0/30, 3.0/40 and 4.5/40, respectively. Hence, the OLR of 3.0 kg $m^{-3} \cdot d^{-1}$ was investigated at both 30 and 40 days HRT. At the maximum OLR (5.5 kg $m^{-3} \cdot d^{-1}$), where undiluted crops were used, the HRTs were 36 and 35 days for crop mixtures B and BM, respectively. The combination of

OLR/HRT, duration of run, TS concentration in feedstock and nutrient addition are presented in Table 2. The crop mixture BMT was not investigated at the final load due to process failure. The feedstocks were fed manually in duplicate reactors through a sampling port in the lower half of the reactors, from which samples of the effluent were also manually withdrawn. To effectuate feeding of the semi-solid crop mixtures without water addition (at the final OLR), a portion of the reactor liquid was withdrawn, mixed with the crop sample and nutrients, and then fed to the reactors.

Antifoam (silicone 414, Savern & Werner) was added frequently to reduce foaming. The experiment was run for 429 days, as indicated in Table 2. Samples were collected daily to determine biogas volume, methane content and pH. Samples for the determination of NH_4-N, alkalinity, TS/VS and volatile fatty acids (VFAs) were withdrawn at the end of each combination of OLR/HRT investigated, or more frequently when the processes showed signs of instability. All sampling was carried out immediately before feeding. The biogas produced was collected in airtight gas collection bags (Transofoil, Flextrus, Sweden) through gas-tight tubing (Saint-Gobain PP, USA). TS-based methane yields were determined, and the values for the last 14 days at each combination of OLR/HRT were analysed.

2.2.3 Analytical methods

TS, VS and pH were determined according to standard methods [22]. Biogas composition was determined using gas chromatography, as described previously [23]. The total gas volume was measured using a graduated 100-mL gas-tight glass syringe with a sample lock (Fortuna, Germany) in the BMP tests, and a wet-type gas meter (Schlumberger,

Karlsruhe, Germany) for the CSTR experiments. Methane yields were calculated as the net amount of methane produced per unit TS added to the reactors, and normalized by correcting the temperature to 273 K, assuming a constant pressure of 101.3 kPa. VFAs were analysed with HPLC (Varian Star 9000, Varian, Walnut Creek, CA, USA), using a Biorad column (Cat. 125-0115, Hercules, USA), as described previously by [23]. The NH_4-N concentrations were measured with the Dr Lange LCK 303 kit (Dr Bruno Lange GmbH, Dusseldorf, Germany) after diluting a 0.45 μm filtered sample to lie within the detection range. C and N were analysed by elementary analysis, S, P and Fe were analysed using ICP-OES and Ni, Mo and Co were analysed using ICP-MS by LMI AB (Helsingborg, Sweden). The analyses were performed after acid digestion, and on one occasion after filtration.

2.2.4 Statistical analysis

Grubb's test ($P \leq 0.05$) was used to check for outliers in the triplicate BMP tests. Analysis of variance ($P \leq 0.05$, one-way ANOVA) and Turkey's multi-comparison test were performed with the statistical package SPSS, version 16. The ANOVA was conducted to compare mean methane yields between BMP and CSTR experiments. The mean methane yields at different OLRs within and between the different CSTR processes were also compared.

Prior to the ANOVA analysis, the relative standard deviation (RSD) (n = 14) was used to measure the spread in the duplicate CSTR experiments. The standard deviation (SD) was pooled in the BMP test from the triplicate test samples and inoculum. In the CSTR, SDs were pooled from within reactors and between reactors (duplicates) except for the process run with crop mixture B at OLR of 4.5 and 5.5 kg $m^{-3} \cdot d^{-1}$ where the SD was that of

the 14 last measurement in a single reactor. The SD was also determined for pH and methane content.

3 Results and Discussion

3.1 Operational conditions and nutrient concentrations

The characteristics and nutrient concentrations analysed for the different crop mixtures and in the inoculum are given in Table 1. The nutrients listed in Table are those found (after chemical analyses) to be limited in the crop mixtures. The nutrient concentrations in the inoculum, collected from an active biogas plant, were higher than those in the crop mixtures. Crop mixture BMT with a high amount of cereal had higher concentrations of N and P as compared to B and BM. Macro- and micronutrients were added in this study because the crop mixtures were not deemed to meet the basic nutrient requirements for anaerobic digestion, and by virtue of the fact that the crop mixtures were diluted to obtain the desired OLR at constant HRT during the experiment. The amounts of nutrients added were calculated based on the intrinsic nutrient concentrations in the BM mixture. The same amounts of nutrients were then added to all crop mixtures. The amounts of nutrients added and the TS of the feedstock at the different OLR/HRT are given in Table 2. The aim was to keep the total nutrient concentration (the sum of the intrinsic nutrients in the crops, Table 1, and nutrients added, Table 2) constant in the feedstock. In other words, the addition of nutrients was reduced as the crop concentration increased in the feedstock. This was, however, not the case at change in level from (OLR/HRT) 1.5/30 to 3.0/30 and from 3/30 to 3.0/40. When increasing the OLR from 1.5 to 3.0 kg $m^{-3} \cdot d^{-1}$, the concentration of N added was increased from 1670 mg L^{-1} to 2500 mg L^{-1} to improve process stability (Table 2). When increasing the HRT

from 30 to 40 days, the nutrient concentration was kept constant, which in fact meant a decrease in addition, since the amount of feedstock added was decreased from 100 g to 75 g per day to achieve the change in HRT. Thereafter, the original plan was maintained, and as the crop concentration increased in the feedstock, the addition of nutrients was decreased.

Table 1. TS, VS, C and mineral concentrations in the investigated crop mixtures and inoculum.

	B	**BM**	**BMT**	**Inoculum**[a]
TS (%)	19.7	19.0	21.3	3.2
VS (% of TS)	93.6	95.5	95.6	1.9
C (% of TS)	40.7	41.5	42.6	n.a.
Nutrient content (mg kg^{-1})[b]				
N	2307	2709	3068	4421
P	291	353	435	500
S	169	190	196	428
Fe	37	34	24	852
Co	0.05	0.04	0.03	0.09
Mo	0.03	0.04	0.09	0.10
Ni	0.10	0.12	0.10	0.38

[a]The inoculum concentrations are the average of duplicate samples.

n.a.: not analysed.

[b]The nutrient concentrations are given in mg/kg wet weight of the crops and are the average of triplicate measurements.

The selected micronutrients (Fe, Co, Ni and Mo) have been reported to be beneficial in anaerobic digestion in several studies [1,12, 16, 24, 25]. The concentrations were selected to be in the higher limit of what has

previously been reported to be stimulatory to the biogas process, as summarized by [13].

In the report by FNR[7], the 13 crop based biogas plants with more than 50% manure addition had on average 2.8 g L^{-1} NH_4-N in the effluent. The nitrogen addition in the present study was set with the ambition to reach a total NH_4-N concentration (added nitrogen plus mineralised nitrogen from the crops) in the reactor of above 2.5 g L^{-1}. This also gave C:N ratios for the feedstock of between 20-26. C:N ratios in this range have been reported to improve process stability and enhance methane yields in the anaerobic digestion of fruit and vegetable waste [26].

Table 2. Macro- and micronutrient addition at the different OLRs (kg $m^{-3} \cdot d^{-1}$) and HRTs (days) investigated. 'd' indicates the number of days at each OLR/HRT. The nutrient concentrations are given in mg kg^{-1} wet weight of the prepared feedstock. TS in refers to the TS in the feedstock after dilution.

OLR/HRT	**1.5/30 (1-73 d)**	**3.0/30 (74-143 d)**	**3.0/40 (144-243 d)**	**4.5/40 (244-366 d)**	**5.5/35-36 (366-429 d)**
TS in (%)	4.5	9.0	12.0	18.0	Undiluted
N	1670	2500	2500	1610	1564
P	389	389	389	269	263
S	385	385	385	268	262
Fe	46.0	46.0	46.0	31.3	30.5
Ni	0.5	0.5	-[a]	-[a]	-[a]
Co	2.0	2.0	2.0	1.9	1.8
Mo	1.6[b]	1.6[b]	1.6	1.5	1.4

[a] After analysis, the Ni level was found to be high probably due to contamination by the equipment, and no more Ni was added.

[b] After analysis, it was discovered that a calculation error had been made for Mo, and the actual addition at the first two loads investigated was 7 times higher than intended.

The content of P in manure is 600-1400 mg L^{-1} [27], while the level in the feedstocks were 290-440 mg kg^{-1} based on ww. P was added to reach the lower limit of the concentration found in manure. The addition of S was in the same magnitude as that of P, based on experiences from [2].

The nutrient concentrations in the effluent were measured on one occasion, on day 121 (OLR/HRT 3.0/30). The total nutrients after acid digestion, and the dissolved nutrients found in the filtrate were compared to assess the solubility as an indication of the bioavailability (Table 3). The

total nutrient concentrations in the effluent were comparable in all the crop mixtures. However, only an average of 15% of the micronutrients was found in the dissolved phase. For S and P, the fraction found in the dissolved phase was 27% on average, and for N, 70-73% was fond in the dissolved phase in the form of NH_4-N. It was also found that the concentration of S was very low compared to the concentration added. A plausible explanation is that S was removed together with biogas in the form of gaseous hydrogen sulphide. Ni concentrations were found to be 10 times higher than expected. This is believed to have originated from contamination by metal parts in the equipment. Since the toxic level of Ni is very close to the beneficial level[11], and excess Ni causes inhibition of methanogenesis [28], Ni addition was terminated at the end of the combination of OLR/HRT 3.0/30 (Table 2). The Ni concentration was highest in one of the BM replicates, i.e. 6.7 mg L^{-1} compared to 4.4 mg L^{-1} in the other replicate, with 1.3 mg L^{-1} and 0.5 mg L^{-1} dissolved Ni respectively. At this stage, the replicate with the higher Ni concentration showed a tendency to decrease in methane production (data not shown). Dissolved Ni above 1 mg L^{-1} have been reported to cause inhibition of methanogenesis [29]. Therefore, the contents of the two BM replicate reactors were mixed and the process restarted at a combination of OLR/HRT of 3.0 kg $m^{-3}\cdot d^{-1}$/40 days. It was also discovered that a calculation error was made for Mo (making it 7 times higher) at the first two loads which was later corrected. Although the concentration of Mo was high, Mo seems to have a higher inhibitory threshold than Ni. Mo has been shown to stimulate methane production over a wide range of concentrations from 0.16 mg L^{-1} to 49.9 mg L^{-1}[11].

Table 3. Effluent TS and nutrients measured as total nutrients and dissolved nutrients (indicated by *) at 3.0 kg $m^{-3} \cdot d^{-1}$ and a HRT of 30 days. The values are averages of duplicate measurements.

	B	B*	BM	BM*	BMT	BMT*
TS (%)	2.3		2.9		3	
			(mg kg^{-1})			
N	3500	2540[a]	3700	2710[a]	3700	2590[a]
P	360	93	415	103	480	210
S	150	39	185	36.5	180	40
Fe	67	3.4	75.5	6.45	66	6.3
Co	2.4	0.2	2.8	0.4	2.8	0.4
Mo	16	2.1	15	6.8	17	2.1
Ni	4	0.5	5.55	1.3	4.5	0.4

[a] The dissolved nitrogen was analysed as ammonia-nitrogen.

3.2 Methane yields

Process performance and conversion efficiency were evaluated in terms of methane yields. Figure 1 show the methane yields achieved in both the CSTR and BMP experiments. High methane yields were achieved in both cases, with values ranging from 350 to 412 m^3 CH_4 kg^{-1} TS added. Turkey's multiple comparison tests, using one-way ANOVA, of the CSTRs results showed that:

(1) The methane yields for the crop mixtures B, BM and BMT processes did not change significantly as the OLR was increased during stable operation. In other words, the methane yields from the digestion of crop mixtures B and BM were comparable up to an OLR of 4.5 kg m^{-}

$^{3}\cdot d^{-1}$, while the yields from the digestion of the BMT mixture were comparable up to an OLR of 3.0 kg $m^{-3}\cdot d^{-1}$.

(2) The methane yields compared between the various feedstocks did not differ significantly. That is, under stable operation, the methane yields for B, BM and BMT processes compared with each other did not differ significantly at the various OLRs/HRTs. Hence, feedstock composition did not affect the TS-based methane yields during stable operation.

These results show that macro-and micronutrient addition enabled stable operation and high methane yields at relatively short HRTs. Stable operation and high methane yields have also be reported at short HRTs in a previous study where macro-and micronutrients addition was employed [30]. The findings in the present study are in contrast to those from energy-crop-based anaerobic digestion in Germany, where micronutrient addition is common [3], but the HRTs are still kept very high when little or no manure is added (FNR [7]). Weiland [3] reiterated that it was necessary to add micronutrients even in processes with 50% share of manure to improve degradability. The above postulation is in line with the present study where not only micronutrients but macro-and micronutrients were added to improve the overall process performance.

The maximum expected methane yields from the different crop mixtures were generated in the BMP experiments, and the methane yields from the CSTR experiments were evaluated based on the BMP yields. The BMP yields ranged from 391 to 411 m^3 CH_4 kg^{-1} TS, with crop mixture B (whole sugar beet plant) showing the highest yield (Figure 1). Analysis of variance (95% confidence interval) showed no significant difference between the BMP yields for the different crop mixtures. Methane yields achieved during stable operation in the CSTRs were on average lower, but not

significantly different from the respective BMP yields. At the highest stable OLR, on average 87%, 90% and 89% of the BMP yields could be achieved in the CSTR processes for the crop mixtures B, BM and BMT, respectively. The BMP yields were similar to those previously reported for individual fresh crop samples [6, 31-32].

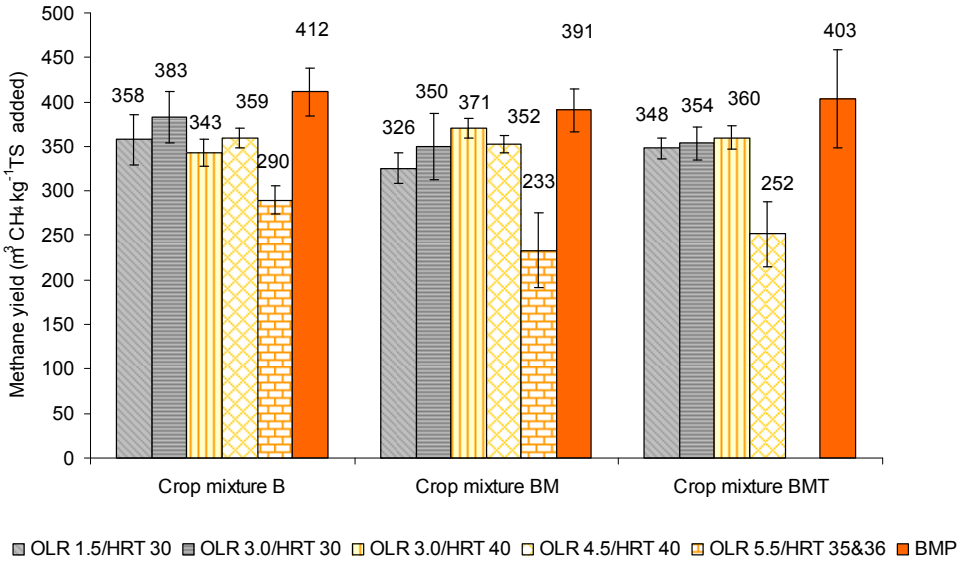

Figure 1. Methane yields obtained under different experimental conditions (OLR and HRT) for the different crop mixtures. The error bars show the standard deviations, as described in materials and methods.

At the final OLR investigated, the methane yields decreased significantly, or process failure occurred with all feedstocks. At this stage, an interesting difference was observed between the different feedstocks. For the crop mixture BMT, the process failed when the OLR was increased from 3.0 to 4.5 kg $m^{-3} \cdot d^{-1}$ on day 286. At the next increase in OLR, from

4.5 to 5.5 kg $m^{-3} \cdot d^{-1}$, the BM fed process failed on day 393. At an OLR of 5.5 kg $m^{-3} \cdot d^{-1}$, the process fed with B showed lower but stable methane yields until the process was stopped on day 429.

3.3 Process stability

Process stability in this study was evaluated in terms of VFAs, the ratio of VFAs to alkalinity (β), pH and methane content (Figure 2). These parameters showed differences in the processes using different feedstocks, especially at the final OLRs. The stability of the processes with the different feedstocks was ranked as follows: B>BM>BMT; the digestion of whole sugar beets only (B) being the most stable process.

3.3.1 VFAs

The total VFAs ranged from 0.3 to 6.5 g L^{-1}, and increased with increasing OLR (Figure 2a). However, the total VFAs at an OLR of 1.5 kg $m^{-3} \cdot d^{-1}$ (at start-up) were higher than those at an OLR of 3 kg $m^{-3} \cdot d^{-1}$ for all processes. This could have been due to the acclimatisation of the microorganisms (inoculum) to the carbohydrate-rich feedstocks and the environmental conditions. The concentration of N in the feedstocks was also increased as the OLR was increased from 1.5 to 3.0 kg $m^{-3} \cdot d^{-1}$ (Table 2). This probably enabled greater stability in the processes despite the higher OLR. In the digestion of crop mixture B, the total VFAs were less than 1 g L^{-1} up to the final OLR. The total VFAs were above 2 g L^{-1} in the digestion of the BM mixture, and above 6 g L^{-1} in the BMT mixture. Increased concentrations of propionic acid were observed at the final operational OLR in the BM and BMT experiments (Figure 2a). The accumulation of propionic acid was the most noticeable difference in the

digestion of the BMT mixture, showing distinctly higher concentrations (3.8 g/L). In a well-balanced anaerobic digestion process, VFAs levels are usually low [33]. The propionic acid concentration has been used as an indicator of process stability; propionate concentrations above 1 g L^{-1} indicating process failure [18]. Propionic acid accumulation in this study, especially in the crop mixture BMT probably meant that propionate degrading acetogens or hydrogenotrophic methanogens were inhibited [18] resulting in process failure. Ammonia, a component of NH_4-N could not have been the reason for the accumulation of propionic (results not shown); NH_4-N values in this study ranged from 0.7 to 2.6 g L^{-1} throughout all experiments with all feedstocks. Concentrations of NH_4-N in the range mentioned above have been reported to be beneficial in the anaerobic digestion process [33].

The cause of the accumulation of VFAs, especially propionic acid in the crop mixture BMT, was not determined in this experiment. Increased concentrations of propionic acid have previously been reported as a consequence of organic overload in CSTRs [34]. However, in the present study, it is more likely to be an effect of feedstock characteristics, as no propionic acid accumulation was seen in the experiments with crop mixture B (Figure 2a). The accumulation of propionic acid may be the result of phytic acid present in cereals such as triticale and maize [35] (in the BM and BMT mixtures), although this was not evaluated in this study. Phytic acid has the tendency to chelate metal ions such as Fe, Ni, Co and Mo (i.e. micronutrients) and macromolecules such as proteins and starch [35]. It has also been reported to inhibit methanogenesis [36]. Although micronutrients such as Fe, Co, Mo and Ni have been reported to promote propionate degradation [16] and also aid hydrogenotrophic metabolism, these micronutrients could have been chelated in the present study by

phytic acid, resulting in impairment of the absorption of these metal ions. This may have resulted in poor bio-availability of the Fe, Ni, Co and Mo added.

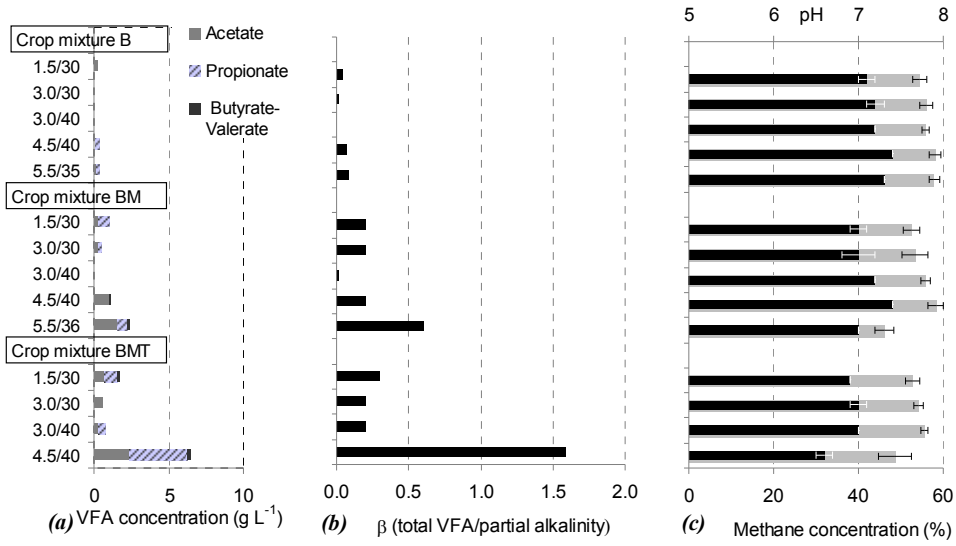

Figure 2. Parameters analysed for the three crop mixtures (B, BM and BMT) at the OLRs and HRTs investigated, as indicated by OLR/HRT on the y-axis. (a) Volatile fatty acids (VFA) where C4/C5 are butyric and valeric acids. (b) β (the ratio between total VFAs and partial alkalinity). (c) pH (upper axis, black bars) and percentage methane in biogas (lower axis, grey bars). The error bars show the standard deviations.

3.3.2 Ratio of VFAs to alkalinity

The ratio of VFAs to alkalinity (β) followed the same trend as the VFAs, with values of less than 0.5 in the digestion of feedstock B, above 0.5 in the BM mixture and above 1.5 in the BMT mixture (Figure 2b). Stability in anaerobic processes has been expressed in terms of β in previous studies [18, 26], with values below 0.5 indicating no process inhibition or

acidification. This value was obtained in the experiments on crop mixture B; in the BM experiments from an OLR of 1.5 to 4.5 kg $m^{-3}\cdot d^{-1}$, and in the BMT experiments from an OLR of 1.5 to 3 kg $m^{-3}\cdot d^{-1}$. Experiments on crop mixture BM at an OLR of 5.5 kg $m^{-3}\cdot d^{-1}$ showed β values greater than 0.5 which led to process instability [18]. At β values above 0.8, significant process instability has been shown to occur, leading to process failure. This was observed in this work, in the experiments on the crop mixture BMT at an OLR of 4.5 kg $m^{-3}\cdot d^{-1}$ (Figure 2b).

3.3.3 Methane content and pH

Figure 2c shows the variation in the methane content in the biogas and the pH during the study. The methane content achieved during stable operation ranged from 52% to 58% and was highest in the digestion of whole sugar beets (mixture B). The methane contents in this study are in agreement with values commonly reported for anaerobic single-stage digesters [26] and for fresh carbohydrate-rich substrates[32]. During unstable conditions, the methane content decreased to values between 46% and 48%. Although the methane yield from sugar beets alone was low at the final OLR (Figure 1), the methane content remained high (57%).

The pH in the reactors ranged from 6.6 to 7.4 (6.9 to 7.4 during stable operation) and increased when N addition (buffering capacity) was increased in the feedstock (Figure 2c and Table 2). The addition of buffering capacity to the feedstock maintained the pH within the range favourable for methanogenesis (6.8 to 7.2) [33]. In the present study, pH values above 6.8 and methane content above 50% were considered indicative of stable processes and hence active methanogenesis. This was however not the case for crop mixture B process at the final OLR, the

above conditions were fulfilled, but there was a significant decrease in methane production.

3.3.4 Foaming

Foaming was observed during the CSTR experiments, especially in crop mixture B, and as the OLR was increased. This was probably the result of the rapid fermentation of easily hydrolysable sucrose in the sugar beets[32]. This produced large amounts of carbon dioxide and VFAs, which reduces the surface tension of the reaction liquid, hence inducing foaming [12]. The addition of N in the form of bicarbonate could also have increased the production of carbon dioxide in the reactors. The foaming episodes were most prominent just after feeding. Foaming fouled the gas collection tubes of one reactor in the digestion of crop mixture B on day 233, leading to a pressure build-up in the reactor, which resulted in the expulsion of the reactor content. However, the duplicate CSTR experiments gave highly reproducible results at a 95% confidence interval, with RSDs ranging between 1 and 3%. Therefore, the continued digestion of crop mixture B in a single reactor after day 233 was considered to give a representative result. Foaming was combated by the frequent addition of antifoam and, at times, by a short-term increase in stirring. Also, feeding was performed once a day in the present study, and multiple feeding could have reduced the foaming intensity.

4 Conclusions

Anaerobic digestion of nutrient-supplemented energy crop mixtures (sugar beets, maize and triticale) resulted in high methane yields and stable processes at relatively short HRTs (30 to 40 days). The addition of selected nutrients can thus have the same stabilizing effect as nutrient-rich

substrates such as manure, and long HRT is not a prerequisite for high methane yields from energy crops. The CSTR methane yields were comparable to batch specific methane yields. The digestion of sugar beets alone was most effective, while the feedstock with triticale was least effective. Hence, OLR as well as feedstock composition affected process performance and stability.

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Paper III

Stable operation during pilot-scale anaerobic digestion of nutrient-supplemented maize/sugar beet silage

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Abstract

Biogas production from maize/sugar beet silage was studied under mesophilic conditions in a continuous stirred tank reactor pilot-scale process. While energy crop mono-digestion is often performed with very long hydraulic retention times (HRTs), the present study demonstrated an efficient process operating with a 50-day HRT and a corrected total solids (TS_{corr}) based organic loading rate of 3.4 kg/m^3·d. The good performance was attributed to supplementation with both macro- and micronutrients and was evidenced by good methane yields (318 m^3/ton TS_{corr}) which were comparable to laboratory maximum expected yields plus low total volatile fatty acid concentrations (< 0.8 g/L). A viscoplastic and thixotropic digester fluid behaviour was observed, and the viscosity problems common in crop mono-digestion were not seen in this study. The effluent also complied with Swedish certification standards for bio-fertilizer for farmland application. Nutrient addition thus rendered a stable biogas process, while the effluent was a good quality bio-fertilizer.

Keywords: Bio-fertilizer; Biogas; Energy crops; Macronutrients; Micronutrients; Stability

Introduction

The use of energy crops as feedstock for biogas production is increasing. An estimated 7% of the farmland in Germany was used for this purpose in 2011 (FNR, 2012). The characteristics of crops, such as poor nutrient concentration, have led to problems such low methane yields, acidification and process instability in crop mono-digestion, leading to application of low organic loading rates (OLRs) and long hydraulic retention times (HRTs) (Lebuhn et al., 2008; Weiland, 2010). A deficiency of micronutrients such as iron (Fe), cobalt (Co), nickel (Ni), tungsten (W) or molybdenum (Mo), has been shown to cause problems in the microbial degradation chain, while supplementation has been shown to improve the performance of anaerobic digestion of crop silage (Hinken et al., 2008; Pobeheim et al., 2010). These inadequate concentrations of micronutrients is one reason why micronutrient supplementation has become more common over the years (Takashima et al., 2011). These micronutrients are cofactors of enzymes or coenzymes involved in the biosynthesis of methane and the growth of anaerobic bacteria. Macronutrients such as nitrogen (N), sulphur (S) and phosphorus (P) also play a vital role in the growth and metabolism of anaerobic bacteria (Pobeheim et al., 2010). Benefits of N in balancing the carbon (C):N ratio and as a buffering agent have been reported (Procházka et al., 2012), and S and P have been reported to boost biogas production as they are components of proteins involved in the biochemistry of biogas production (Takashima et al., 2011).

Thus, adequate amounts of both macro- and micronutrients, as for example in manure (Bruni et al., 2010), are crucial for the overall performance of the biogas process . The requirements for a better nutrient balance are often fulfilled by co-digesting energy crops with manure (Cavinato et al., 2010). However, there is regional scarcity of manure (Lebuhn et al., 2008). In a large number of crop-based biogas plants monitored in a German study (FNR, 2010), presented data showed that continuously stirred tank reactors (CSTRs) operating with more than 50% manure together with the energy crops (13 plants) were operated at an average HRT of 46 ± 9 days, while plants operating with no or less than 30% manure (13 plants) applied an average HRT of 170 ± 58 days. Prolonging the HRT in these plants did not seem to influence the residual methane production (post-digestion at 37 °C), which was 5.2 ± 1.9 m^3 for the plants using little or no manure, per ton of effluent or digestate, and 5.9 ± 1.8 m^3 for the manure-dominated plants (FNR, 2010). This indicates a limitation on substrate biodegradability that is not overcome by prolonged HRT.

Another problem observed in the mono-digestion of energy crops is high total solids (TS) concentrations in the digestate in the processes reviewed by FNR (2010). Furthermore, the crop-based digestate was glutinous in nature, leading to higher viscosity and potential problems with stirring, in contrast to the manure-based digestate. Stressed microbes have however, been reported to promote the synthesis of extracellular polymeric substances (EPS) (Sutherland, 2010), which might be another explanation to increased viscosity of the digestate. Accordingly, in a crop mono-digestion process it is important to characterise rheological parameters such as dynamic viscosity, shear stress, and shear rate to provide information regarding fluid behavior and resistance during mixing (Pevere et al., 2005).

Ensiling is a common means of preserving crops for use as feedstock in biogas production, but reporting methane yields from ensiled crop-based biogas plants can be problematic. Methane yields are usually reported on TS or volatile solids (VS) bases, and these are determined by a method involving oven drying. The presence of volatile compounds in the silage will cause an analytical error in the determination of TS and VS, as has been pointed out by Kreuger et al. (2011). This error will cause overestimation of the reported methane yield, thus many of the published methane yields from crop silage in scientific literature may thus be unreliable (FNR, 2010; Vervaeren et al., 2010). Hence, the performance of a crop-based biogas process using silage must be evaluated using the correct TS values.

It was hypothesized in this study that supplementation with both macro- and micronutrients would allow good biodegradation of energy crop silage at HRTs which are normally only applicable to the co-digestion of crops and manure. Co-ensiled maize and sugar beets were digested in a one-stage pilot-scale CSTR process under mesophilic conditions. The characteristics of the silage were carefully analysed during the study. The process was evaluated by stepwise increases in the OLR at constant HRT of 50 days. Process performance was evaluated in terms of methane yields, volatile fatty acid (VFA) concentration, digestate VS content, residual methane production, rheological characteristics and EPS. The effect of nutrient addition on the digestate properties as a bio-fertilizer was also evaluated according to the Swedish certification scheme SPCR 120 (SP, 2010).

2 Materials and methods

2.1 Substrate and inoculum

The substrate consisted of maize (*Zea mays*) and sugar beet (*Beta Vulgaris*) harvested in October 2009 in southern Sweden. About 20,000 kg was ensiled in layers at a ratio of 1:1 (based on wet weight, ww) in a bunker silo for 8 months prior to the study. The maize was cut at harvest with a precision cutter to sizes ranging from 2 to 5 cm, while sugar beet was crushed to sizes ranging from 1 to 10 cm. Values of TS and VS, and concentrations of ethanol, lactic acid and VFAs were analysed continuously during the study. Eleven samples were collected, on different occasions, from the top to the bottom of the silage pile and homogenized. Two samples were also used for macro-and micronutrient analysis. The inoculum for the study was taken from a biogas plant digesting rest products from sugar production (Nordic Sugar, Örtofta, Sweden). The inoculum had an average pH of 7.6, TS of 14.2%, VS of 2.4% and partial alkalinity of 5.6 g/L.

2.2 Nutrient supplementation

The macronutrients N, P and S, and micronutrients Fe, Co, Mo and Ni were added as salts during the preparation of the feedstock. N was provided by urea ($CO(NH_2)_2$), ammonium chloride (NH_4Cl), ammonium hydrogen carbonate (NH_4HCO_3) and ammonium sulphate ($(NH_4)_2SO_4$) (37%, 25%, 25% and 13%, respectively). $(NH_4)_2SO_4$ also provided S. P was added in the form of sodium hydrogen phosphate ($NaH_2PO_4 \cdot 2H_2O$). Fe was added in the form of iron sulphate ($FeSO_4 \cdot 7H_2O$), Co as cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), Ni as nickel nitrate ($Ni(NO_3)_2$) and Mo as ammonium

molybdate ($(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$). All chemicals were of reagent grade, and were obtained from a commercial source (Savern & Werner, Sweden).

2.3 Biochemical methane potential tests and residual methane potential

The silage was digested in triplicate biochemical methane potential (BMP) tests under mesophilic conditions for 30-33 days, as reported elsewhere (Nges et al., 2011). Four different BMP tests were conducted on silage samples taken during the periods of the four investigated OLRs during the pilot-scale experiment. Samples (300 mL) of the digestate at each OLR were incubated in triplicate at 37 °C ± 1 °C and 23 °C ± 1 °C for 30 days to evaluate the residual methane potential of the digestate.

2.4 Feedstock preparation and storage

The feedstock was prepared weekly, batch-wise, by feeding silage along a conveyer belt to a mixing tank equipped with a rotating-cutting blade, where it was mixed with water and/or digestate and nutrients (Figure 1). Four loads (1-4) were investigated at constant HRT of 50 days (Table 1). The silage was diluted with water at the first three loads, while at the final load, no water was added. At load 4 and during the final phase of load 3, a volume of digestate equal to the volume of feedstock was pumped from the digestate tank to the mixing tank to give a pumpable feedstock. Each batch of prepared feedstock was stored for up to one week, and was semi-continuously fed to the digester. The feedstock at each load was incubated under controlled conditions to establish whether degradation had occurred during storage in the mixing tank. Samples, 300 g, were incubated in

triplicate-500 mL shake flasks for 7 days, at 10 ± 1 °C and 18 ± 1 °C, simulating the preparatory room temperature changes during cold periods (winter) and warm periods (summer), respectively. The flasks were stirred once a day, and the volume of gases (H_2, CO_2 and CH_4) was determined.

2.5 Operation of the pilot-scale process

The study was performed in a single-stage anaerobic digester, CSTR, with a total volume of 2.2 m^3, which was initially charged with 1.8 m^3 of inoculum and maintained at 37 $^{\circ}$C± 1°C. The set up was mounted as previously reported by Bohn et al. (2007), additionally, a digestate tank was introduced for temporary digestate storage (Figure 1). The digester was fed semi-continuously once a day and continuously stirred at 6.8 Hz. The daily feed was 36 kg while 34 kg was discharged. The 1.8 m^3 level in the digester was monitored with the aid of level sensors. The amount fed into the digester was controlled by weighing, while the amount of digestate discharged was controlled by pump flow/discharge time.

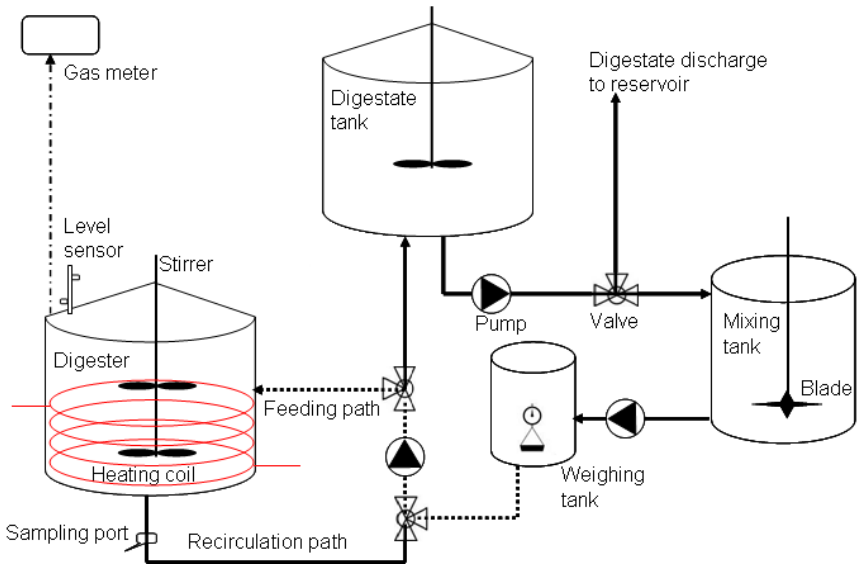

Figure 1. Schematic illustration of the pilot-scale anaerobic digestion process.

A complete feeding cycle consisted of the following steps: (a) simultaneous recirculation of the digester content through the recirculation path and mixing of feedstock in the mixing tank; (b) discharge of digestate to the digestate tank or an outside reservoir; (c) pumping of the feedstock to the weighing tank; (d) pumping of the feedstock to the digester through the feeding path; and (e) recirculation of the reactor content to ensure emptying of the feeding pipes (See Figure 1).

The process was run for a total of 388 days, i.e. load 1 for 140 days, load 2 for 104 days, load 3 for 70 days and load 4 for 74 days. At loads 3 and 4, settling of grit in the digester occasionally led to clogging of the pump upon digestate discharge, causing the automated feeding to stop. At load 4

clogging was encountered on days 335, 359, 368, 371 and 379 (i.e. 5 days out of the 74 days run). The amount of feed was doubled (tripled on one occasion) to compensate for missed feeding.

Samples were taken from the digester before feeding, three times a week. Gas samples were collected from the gas meter through a gas-tight tube, while liquid samples for pH, ammonia nitrogen (NH_4-N), alkalinity, TS, VS and VFA determinations were withdrawn from the sampling port after 3 minutes' internal recirculation. Samples for residual methane determination and nutrient analysis were taken at the end of each load, while samples for viscosity and EPS were withdrawn at the end of load 3 and load 4 only.

2.6 Analytical methods

Feeding rate, temperature and raw biogas volumes were monitored on-line using a SLC5/05 PLC system as described by Bohn et al. (2007). Methane content was determined using gas chromatography with a thermal conductivity detector, as described elsewhere (Nges et al., 2011). Volatile compounds in the silage were determined as described by Kreuger et al. (2011). TS and VS were determined by oven drying (APHA, 2005). Alkalinity and NH_4-N were determined as described previously (Nges et al., 2011). N was analysed using elementary analysis: S, P, potassium (K) and Fe by inductively coupled plasma optical emission spectrometry and Ni, Mo, Co, lead (Pb), chromium (Cr), copper (Cu), mercury (Hg), cadmium (Cd) and zinc (Zn) with inductively coupled plasma mass spectrometry (LMI AB, Helsingborg, Sweden). The analyses were performed after acid digestion of the whole sample according to Swedish

standard SS028311, and of the liquid phase after filtration (V150, Munktell, Sweden) with a pore size of 8-10 μm.

The TS of the silage was corrected for the loss of volatiles (denoted TS_{corr}) according to (Kreuger et al., 2011). The methane yield was determined as dry methane normalised to 0 °C at 101.3 kPa, and was calculated by dividing the volume of methane produced by the amount of feedstock (ww) or TS_{corr} added to the digester. Nutrient concentration in the digestate was calculated based on nutrient concentration in the feedstock and mass loss during digestion. The mass loss was calculated by the ideal gas law using the measured methane volume, percentage of methane in the raw gas, and assuming that carbon dioxide constituted the remaining biogas.

2.7 Assessment of fluid behaviour

Viscosity of the digester fluid was determined using a rotational rheometer (RheolabQC SN80609650) with a CC27-SN19237 measuring system and a C-LTD80/QC cell, coupled with Rheoplus software (Anton Paar). Rheograms including viscosity curves were obtained with a three-step protocol according to Björn et al. (2012). Measurements were done in triplicates, at 37 ^{o}C, on the same day it was sampled from the digester. The fluid behaviour was interpreted by the flow- and viscosity curves (Schramm, 2000) and the dynamic viscosity, limit viscosity and yield stress were noted. The Herschel Bulkley- and Bingham models were applied in order to transform rheogram data values to the rheological behaviour of the fluids according to (Seyssiecq et al., 2003). EPS was extracted using a cation exchange resin (Dowex® Marathon C, Na^{+} form, Sigma- Aldrich) according to Frølund et al. (1996). The extracted EPS was quantified as

total proteins (PN) and total polysaccharides (PS). Proteins were analysed by a modified Lowry method (Frølund et al., 1996) and polysaccharides with the anhtrone method (Wood et al., 2009). Bovine serum albumin and glucose were used as a protein- and polysaccharide standards, respectively.

2.8 Statistical analysis

Grubb's test was used to check for outliers in the laboratory BMP tests. Analysis of variance (ANOVA) was performed with the statistical package SPSS version 16, to determine statistically significant differences in BMP, the pilot-scale methane yields and to compare the BMP and pilot-scale methane yields. The term significant is thus used only when a statistical test has been carried out giving a P-value ≤ 0.05. The standard deviation (SD) was pooled in the BMP test from the triplicate test samples and inoculum. The SD in the pilot-scale trial was determined for the last 14 days of operation at each OLR investigated, for ww-based methane yields, methane content and pH. The SDs in linear and multiplicative operations were combined according to standard statistical rules (Kreuger et al., 2011).

3 Results and discussion

The results section is divided into four parts. The first describes the characteristics of the feedstock, the second the pilot-scale performance (divided into evaluation of the methane yield and evaluation of parameters monitored in the digester liquid), the third part describes the effect of nutrient addition on the bio-fertilizer composition while the final part presents a summary.

3.1 Feedstock characterisation

The quality of the silage and the prepared feedstock was continuously analysed to allow reliable evaluation of the methane yields, and also to investigate if silage quality deteriorated over time.

3.1.1 Changes during ensiling and feedstock storage

Conventional analysis gave an average TS value of 18.6% ± 2.2% and VS of 15.9% ± 2.9%. The TS of the fresh maize and beet mixture before ensiling was 31.1% containing 28.3% VS. The volatile compounds showed large variations, with the following average values: ethanol 0.84% ± 0.69%, lactic acid 0.83% ± 0.61%, acetic acid 0.59% ± 0.39%, propionic acid 0.03% ± 0.01% and butyric acid 0.03% ± 0.02%. However, the concentrations showed no time-dependent change, and no correlation was found with the position in the silage pile from which the samples were taken. The average total volatiles (2.3%) was considerably lower than the average 4.5% previously observed in laboratory ensiling of maize and beets (Kreuger et al., 2011). The average TS and VS values corrected for losses of volatile compounds during drying (using the average concentrations of volatiles) (Kreuger et al., 2011) were 20.3% ± 2.6% and 17.7% ± 2.3%, respectively. The TS_{corr} value was very low compared to the TS of the fresh sample before ensiling (31.1%). The concentration of volatiles in the silage was also lower than expected, and the organic content (VS) was lower after ensiling. A sample of leachate from the bunker silo floor was collected and found to be high in ethanol, lactic acid and VFAs (data not shown). Ensiling losses were not quantified, but the above findings imply that organic material was lost during ensiling. Mass losses are common in efficient ensiling, but energy losses are usually small (Herrmann et al.,

2011; Kreuger et al., 2011; Pakarinen et al., 2011). In this study, a large mass loss occurred during the first 8 months of ensiling, but there was no sizeable change in silage characteristics during the 13-month-long study. Energy losses during ensiling could be the result of aerobic degradation of organic compounds and loss of leachate (Weissbach, 2009). Weiland, (2010) has reported energy losses of 8 to 20% during ensiling for biogas production.

Table 1. Operational parameters during the pilot-scale study

	Load 1	Load 2	Load 3	Load 4
TS_{corr} in mixer tank (%)	8.6	12.6	16.9	20.3
ww-OLR ($kg/m^3 \cdot d$)	8.5	12.4	16.7	20.0
TS_{corr} -OLR ($kg/m^3 \cdot d$)	1.7	2.5	3.4	4.1
HRT (d)	50	50	50	50

The resulting average TS_{corr} value was used to determine the OLR and TS_{corr}-based methane yields during the study. Table 1 show the investigated OLRs based on TS_{corr} and wet silage in this study.

Incubation of the feedstock from the mixing tank at 10 °C and 18 °C showed minor gas losses after 7 days. When the silage was mixed with water and nutrients, no gas production could be detected. However, when the feedstock was mixed with an equal amount of digestate (towards the end of load 3 and load 4), a methane production of 0.7-1.1 m^3/ton silage was recorded irrespective of temperature. This was 1.0-1.6% of the methane collected during digestion in the biogas process and this loss was considered to be negligible. Relatively large volumes of CO_2 were produced during incubation at 18 °C, corresponding to a 1.4% mass loss. This was also considered to have a negligible effect on the final result.

3.1.2 Biochemical methane potential tests

Figure 2 show the BMP tests and pilot-scale methane yields per ton ww and per ton TS_{corr}. The BMP tests were carried out in parallel with the pilot-scale study; i.e., a BMP test was carried out at each OLR. This was done to determine the maximum expected methane yield from the silage during the study period, and to determine variations in silage quality. Figure 2a & 2b shows the methane yields generated in BMP tests on the fresh substrate and the silage. The ww-based methane yield for the fresh sample was significantly higher than those of the silage; however, the silage (ww) methane yields did not differ significantly during the study (Figure 2a). The average values for the TS_{corr}-based methane yields from the silage were lower than for the fresh sample (Figure 2b), but the differences were not significant. Also, although the average value decreased with time, no significant decrease was seen in the TS_{corr}-based methane yields. These results illustrate the considerable deterioration in silage quality that had occurred during the first 8 months of storage, but no sizeable changes in quality or methane potential were noted during the experiment.

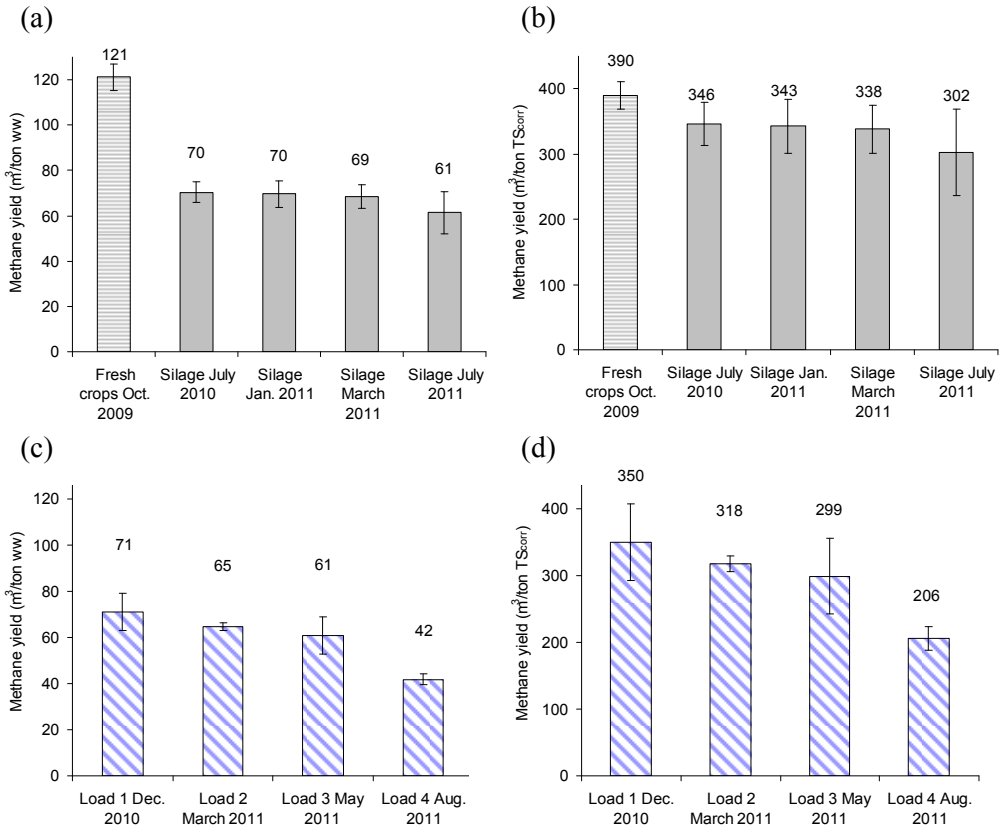

Figure 2. Methane yields from fresh crops and silage determined by BMP tests expressed in (a) ww-basis, (b) TS_{corr}-basis and methane yields from the pilot-scale experiment expressed in ww-basis (c) and TS_{corr} -basis (d).

3.1.3 Nutrient concentrations in the silage and nutrient addition

The concentrations of some of the nutrients in the silage, inoculum and the nutrients added are listed in Table 2. The concentration of added nutrients in the feedstock was kept constant throughout the experiment. The micronutrients Fe, Co, Mo and Ni were chosen since they have previously been shown to be stimulateory in anaerobic digestion processes (Schattauer et al., 2011; Takashima et al., 2011).

Table 2. Concentrations of nutrients (mg/kg) in the silage and inoculum and the nutrients added to the feedstock in the pilot-scale experiment

Nutrient mg/kg	Nutrients in silage	Nutrients in inoculum	Nutrients added to silage
N	2280	2400	2400
P	225	830	420
S	185	530	360
Fe	170	1100	36
Co	0.09	0.92	2.20
Mo	0.11	0.30	1.70
Ni	0.21	1.50	0.50

The total concentrations (added plus indigenous in the silage) of the micronutrients were in the high range of those previously shown to be beneficial in biogas production (Schattauer et al., 2011), but care was taken to restrict the amount of Ni as it is an unwanted heavy metal in the digestate. The positive effects of adding macronutrients to the biogas process have been less well studied. In the present study, N was added to adjust the C:N ratio to give values between 13 (load 1) and 22 (the target at load 4), which is in the range previously reported to improve anaerobic digestion (Bouallagui et al., 2009; Weiland, 2010). Adding nitrogen also increases the buffering capacity of the system, which is important for silage

as the concentrations of organic acids are high (Pakarinen et al., 2011). The amount of P added was based on a suggested optimal N:P ratio of 5 (Speece, 1987), but was decreased to give a target ratio of 7 at load 4. Finally, S was added based on long-term studies by Scherer et al. (2009) in which it was shown that concentrations of S and P in the same range improved the biodegradation of beet silage. S is also a cofactor and component of many enzymes involved in the biosynthesis of methane (Schattauer et al., 2011).

3.2 Process performance

The methane production efficiency of the pilot-scale process was evaluated in three ways: (a) by comparing the methane yields at higher OLRs with yields at lower OLRs, (b) by comparing the pilot-scale methane yields with the BMP methane yields, and (c) by measuring the residual fermentation potential of the digestate at each of the four OLRs. Liquid phase parameters were evaluated to monitor characteristics and changes in the liquid and to assess process stability.

3.2.1 Methane yields

The pilot-scale average methane yields decreased from load 1 to load 4 (Figure 2c & 2d). However, statistical analysis demonstrated that the ww- and TS_{corr}-based methane yields from loads 1 to 3 did not differ significantly, while the ww-based methane yield at load 4 was significantly lower. When giving the methane yields based on TS_{corr}, the pooled standard deviation was high, but the methane yield at load 4 was still significantly lower than at loads 1 and 2 (Figure 2d). The concentration of methane in the biogas also decreased slightly, from 60.1 ± 1.8%, 57.9 ± 1.4% and 57.7

± 0.6% for loads 1-3 respectively, to 53.7 ± 1.4 % for load 4. The high methane content, compared to what is expected for the fresh carbohydrate-rich substrate, can be explained by the loss of carbon dioxide during ensiling in the production of more reduced compounds such as ethanol (Weissbach, 2009).

Comparison of the methane yields in the pilot-scale experiment and the BMP tests showed that the yields based on TS_{corr} did not differ significantly due to the larger standard deviation (Figures 2b & 2d). When ww-based methane yields were compared for the first three OLRs the yields were 101%, 93% and 88% of the BMP results, although there were no significant differences (Figure 2a & 2c). However, the methane yield at load 4 (42 ± 2 m^3CH_4/ton ww) was only 69% of that of the BMP (61 ± 9 m^3CH_4/ton ww), and this difference was statistically significant. Both comparisons show that the increase in TS_{corr}-based OLR from 3.4 to 4.1 $kg/m^3 \cdot d$ led to a decrease in methane yield, and thus in substrate degradation efficiency.

3.2.2 Residual methane production

The residual methane production values determined from the digestate at 37 °C ±1 °C and 23 °C ± 2 °C at each load are presented in table 3. The residual methane production increased as the OLR was increased. However, there was no significant difference in the residual methane production from load 2 to load 4 at 23 °C. At 37 °C, the residual methane production at load 4 was significantly higher than at the other loads, but was still not exceptionally high. The residual methane production at 37 °C in 13 German CSTRs digesting energy crops (mainly maize silage) with 0-

30% manure, has been reported to be 5.2 m^3 CH_4/ton digestate, although the average HRT was as long as 170 days (FNR, 2010).

3.2.3 Liquid phase parameters

Considerable effort should be devoted to obtaining a stable process as this guarantee the successful conversion of organic matter to biogas. Stability in this study was evaluated based on the evolution of VFAs, VS, pH and partial alkalinity in the digester. In addition, both total and dissolved nutrients were measured to monitor the changes over time.

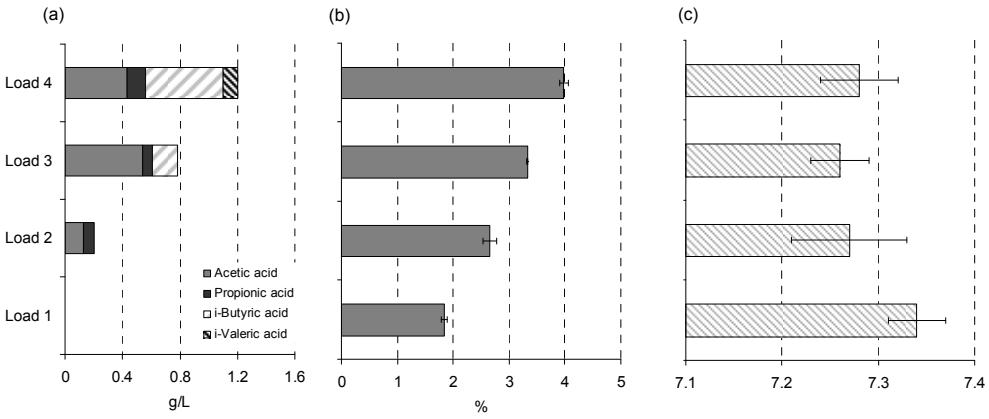

Figure 3. Concentration of total VFAs (a), VS (b) and pH (c), during the entire pilot-scale study. The error bars are SD.

Finally, viscosity was used to study the rheological nature of the digestate as well as EPS concentrations. During the study, nutrient addition was constant for the whole 13 months of operation, while at each load, the concentration of silage in the feedstock was maintained for only 2.5-4.5 months. It would, of course, have been desirable to run each load for a longer time, but the degradation efficiency of the process was deemed to be

stable at the end of each load based on the low fluctuation in biogas production (data not shown). Figure 3 shows the VFAs, VS and pH in the digestate at the four loads. The concentration of total VFAs was low, ranging from 0 to 1.2 g/L, but showed an increasing trend (Figure 3a). The dominating acids were acetic acid at lower loads and increasing butyric acid concentrations at higher loads. Acetic acid is usually present at low concentrations in biogas processes, while the accumulation of propionic and butyric acid are more severe indicators of the inhibition of methanogens (Weiland, 2010). Propionic acid accumulation has been linked to failure in nutrient-poor biogas processes (Takashima et al., 2011). Very little propionic acid was seen to accumulate in the present study, but at load 4, a decrease in the ability of methanogens to convert the VFAs to methane was observed, together with a lower methane yield (Figure 2c & 2d), higher residual methane production (Table 3), higher VS in the digester (Figure 3b) and an increase in EPS concentrations (Table 3). However, addition of N contributed to high partial alkalinity in the process (Table 3), which gave stable pH values (Figure 3c), and a ratio of total VFAs to partial alkalinity below 0.2, indicating a stable, non-acidifying process (Bouallagui et al., 2009; Lebuhn et al., 2008).

Table 3. Values of the parameters monitored during the pilot-scale study

	Load 1	Load 2	Load 3	Load 4
Partial alkalinity (g/L)	4.6 ± 0.1	6.9 ± 0.3	6.0 ± 0.8	6.9 ± 1.1
TS in effluent (%)	5.3 ± 0.1	7.0 ± 0.5	4.93± 0.0	7.9 ± 0.1
VS in effluent (%)	1.8 ± 0.1	2.7 ± 0.1	3.33 ± 0.01	4.0 ± 0.1
Residual methane m^3/ton digestate (23 ^oC)	1.0 ± 0.1	1.3 ± 0.2	1.4 ± 0.2	1.9 ± 0.5
Residual methane m^3/ton digestate (37 ^oC)	1.5 ± 0.1	3.1 ± 0.5	3.3 ± 0.3	4.1 ± 0.4
PN-EPS (mg/L)	nd	nd	3.06 ± 0.01	4.03 ± 0.05
PS-EPS (mg/L)	nd	nd	0.70 ± 0.00	0.96 ± 0.04

nd: Not determined

The VS in the digestate increased gradually with increasing OLR. The decrease in TS at load 3 can be explained by the removal of grit that had settled in the digester towards the end of load 3 (Table 3). The increasing VS content in the digestate (Figure 3b) and the residual methane potential during incubation at 37 °C (Table 3) show good correlation, and both parameters indicate that the increasing OLR led to lower degradation efficiency in the process. In the data presented by FNR (2010), the crop based CSTRs with < 12% manure addition (7 plants) had an average HRT of 182 ± 58 days, and the TS in the digestate was 7.7 ± 1.1 which is the same range as at the final load in this study (Table 3). However, the VS was much higher 5.9 ± 0.8 as compared to 4.0 ± 0.1 at load 4 (Table 3).

There was a significant increase in total EPS from load 3 to load 4. The protein (PN-EPS) and polysaccharide (PS-EPS) concentrations are presented in table 3. Results showed that the PN fraction was higher than the PS fraction as has also been reported for activated sludge (Frølund et al., 1996). Also, the PN/PS ratios (4.3 and 4.2) were similar despite the difference in OLR. The proportion of EPS of VS was 11.3 and 12.5% at load 3 and load 4, respectively. Less EPS (8.6% of VS) has been observed in the anaerobic co-digestion of cow manure and maize silage with OLR of 4.0 $kg/m^3 \cdot d$ (unpublished results) but no published data on EPS in crop based anaerobic digestion has been found, hence further investigations need to be carried out as EPS accumulation might be a reason for the observed high viscosity in nutrient limited digesters. Increased EPS synthesis have been reported as due to limitations in nutrients such as N, P and K which might increase the viscosity of the digester content by the formation of weak or strong gels (Sutherland, 2010).

The total and dissolved nutrients and heavy metals in the digestate are presented in Table 4. Since the bioavailability of nutrients is not always related to the total amount (Pobeheim et al., 2010), dissolved nutrients were measured to give a rough indication of bioavailability. In the present study, the macronutrients were found to a higher extent in the dissolved phase compared to the micronutrients (i.e. on average 58% for N, 18% for P and 21% for S). Dissolved N was measured as NH_4-N, and the highest value found was 2.4 g/L, which is in the same range as that reported for energy crop digestion plants in Germany with over 50% manure addition: on average 2.8 g/L (FNR, 2010). NH_4-N concentrations between 2 and 3 g/L have been reported to provide stability and offer the possibility of using higher OLRs in the biogas process (Procházka et al., 2012). NH_4-N concentrations in this range have been reported to be non-toxic under the

conditions prevailing in the digester (neutral pH and mesophilic conditions) (Chen et al., 2008).

Table 4. Amounts of nutrients in the effluent/digestate at each OLR

Nutrient	Load 1		Load 2		Load 3		Load 4	
(mg/kg)	Total	Dissolve	Total	Dissolve	Total	Dissolve	Total	Dissolve
N*	2590	1520	3370	1875	3620	2136	4040	2393
P	590	nd	730	100	700	110	690	170
S	450	nd	420	40	480	100	460	150
K	720	nd	970	990	1200	1200	1400	1500
Fe	690	nd	630	6.30	540	17	450	34
Co	3.10	nd	2.60	0.08	2.80	0.15	2.20	0.24
Mo	3.30	nd	2.90	0.20	3.60	0.09	3.20	0.30
Ni	0.72	nd	1.00	0.14	1.50	0.20	1.20	0.29

* Dissolved nitrogen was measured only as NH_4-N.
nd: Not determined

The soluble forms of micronutrients increased with increasing OLR, but were in the same range as those reported by (Takashima et al., 2011), i.e., 1% to 9% of the total amount, apart from Ni, for which 24% was in the dissolved form at OLR 4. The concentrations of the micronutrients Ni, Co and Mo in the dissolved form are within the range reported to be beneficial to the anaerobic digestion process (Pobeheim et al., 2010; Takashima et al., 2011). The negative effects of Ni and other heavy metals are discussed in Section 3.3.

3.2.4 Rheological characteristics of the digestate

Rheological behaviour of digester content is of great importance since it affects transports processes and efficient mixing. The rheogram for digester fluid sampled at the highest stable load i.e. TS_{corr} based OLR of 3.4 $kg/m^3 \cdot d$ is presented in figure 4. The rheogram for the reactor fluid at load 4 was very similar, but is not shown since the measurement was not performed until 5 days after feeding was stopped, and the measurement is not seen as representative for a fully loaded process. Decreasing shear stress was initially illustrated, before the exerted shear stress turned more constant. A yield stress of 81 (±15) Pa (the force a fluid overcomes in order to start flowing) was detected, indicating viscoplastic behaviours, i.e. a pseudoplastic behaviour with yield stress according to the definitions by Schramm, (2000). There was a deformation of fluid structure involving a breaking of aggregates at a certain shear rate and this caused a reduction in viscosity. The initial dynamic viscosity at 20 1/s was 4.3 ± 0.1 Pa·s which decreased with increasing shear rate, until it reached its limit viscosity of 0.02 ± 0 Pa·s. The viscosity initially dropped quickly, specifically indicating Bingham viscoplastic fluids with pseudo-Newtonian behaviour. The Herchel-Bulkley and Bingham models also indicated that digester fluid

was viscoplastic, since the yield stress-value was >0 (717 ± 361 and 24 ± 6, respectively).

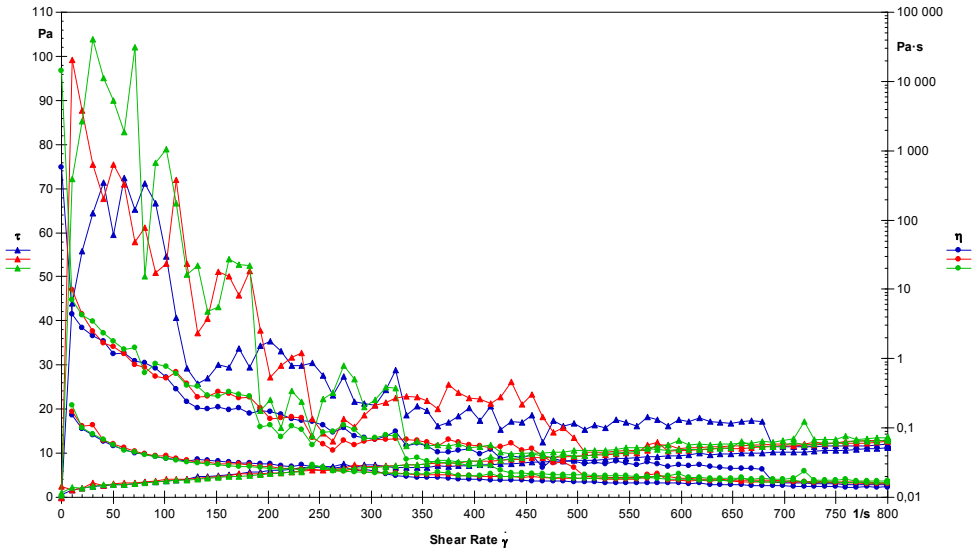

Figure 4. Rheogram – flow (▲,▲,▲) and viscosity (♦,♦,♦) curves for pilot digester fluids sampled at OLR 3.4 kg /m^3·d, with a three-step protocol (triplicate analysis). Flow curves illustrating shear stress (τ; Pa) vs. shear rate (γ; 1/s) and viscosity curves illustrating dynamic viscosity (η; Pa·s) vs. shear stress (γ; 1/s).

A distinct difference between the flow curves was noticeable when the shear rate increased (interval 0 to 800 1/s) and afterwards decreased (interval 800 to 0 1/s; Figure 4). This area describes the degree of thixotropy i.e. the increase of this area is related to the amount of energy required to break down the thixotropic structure (Schramm, 2000). Hence the digestate showed viscoplastic and thixotropic fluid behaviour. Similar findings have been reported for mesophilic processes in laboratory-scale

treating cereal and paper and pulp mill residues (Björn et al., 2012). In opposite to pseudoplastic fluids, the viscosity of thixotropic fluid is time dependent. This means that the structure will be rebuilt once the stirring has stopped and the fluid is at rest (Seyssiecq et al., 2003). Thus, intermittent stirring is not recommended for the investigated process. Thixotropic fluids are affected by intermolecular forces when they are at rest, which turns the liquid into a solid, thus, increasing the viscosity (Schramm, 2000). In this study, the digester was continuously stirred and viscosity problems like those reported for the mono-digestion of energy crops (FNR, 2010) were not seen.

3.3 The effect of nutrients on bio-fertilizer quality

The guideline values for heavy metals in bio-fertilizer according to SPCR 120 are given in Table 5 (SP, 2010). These are the same as the limit values for the application of sewage sludge as a bio-fertilizer in agriculture (SJV, 2010). The amount of bio-fertilizer that can be added is limited by the soil class, which in southern Sweden means an average annual addition of 22 kg P/ha over 5 years, and 150 kg/ha for easily available N (SJV, 2010). Table 5 shows the amounts of digestate that can be added per ha to reach 22 kg P/ha, and the amounts of desirable nutrients and undesirable heavy metals resulting from this addition. The amounts are presented for three cases: a) calculated concentrations without any nutrient addition, b) calculated concentrations with the nutrient addition used in this study, and c) actual concentrations based on measurements on the digestate (given in Table 4). The calculations were performed as outlined in Section 2.7, and based on the conditions/performance at load 3 (OLR of 3.4 kg/m^3·d) since the process showed good efficiency and stability at this OLR.

For the silage investigated here, the low contents of N and P make the digestate less interesting as a bio-fertilizer. Furthermore, the guideline values for Pb and Cd are exceeded (shown in italics in Table 5). The high Cd:P ratio in the silage causes the amount of Cd added per ha to exceed the guideline value by a factor of 3.5. If no macronutrients are added, the amount of digestate required to reach the desired fertilization levels for P and N is very high. This will make transport and spreading costs high, and the digestate less attractive as a bio-fertilizer. In this study, P was added in order to obtain a Cd:P ratio that would allow the digestate to comply with certification schemes (0.75 g/ha), but the actual value calculated after the experiment was slightly over the limit (0.81 g/ha). Care was taken when adding Ni so as not to exceed the maximum permitted level in the bio-fertilizer. It is worth noting that if P had not been added as well, Ni would have exceeded the limit values.

Table 5. Amounts of digestate that can be added per ha to reach 22 kg P/ha, and the effect of this on the amounts of nutrients and heavy metals added

	Guideline/limit values	Calculated, without nutrients*	Calculated, with nutrients	Measured, with nutrients
Digestate addition (ton/ha)		105.3	32.4	31.4
Nutrients (kg/ha)				
N-tot		223	156	114
NH_4-N	**150**	90	82	67
P	**22**	22	22	22
K		96 (985)	30	38 (540)
S		18	19	15
Heavy metals (g/ha)				
Ni	**25**	24 (0.21)	20	47 (1.50)
Pb	**25**	*30* (0.31)	9	13 (0.40)
Cr	**40**	34 (0.35)	10	35 (1.10)
Cu	**300**	210 (2.15)	65	104 (3.30)
Hg	**1**	0.7 (0.01)	0.2	0.2 (0.01)
Cd	**0.75**	2.64 (0.03)	*0.81*	0.75 (0.02)
Zn	**600**	333 (3.40)	102	408 (13.00)

*Values in brackets are concentrations determined in the silage and digestate expressed as mg/kg. These concentrations are given for N, P and S in Table 2 for the silage and in Table 4 for the digestate

The calculated and measured values did not agree in all cases. The measured ammonia concentration is lower than the calculated value, which could partly be explained by loss of NH_3 in the biogas. Also, the measured amounts of some heavy metals exceeded the calculated values. Increased amounts of Cr, Ni, Zn and Fe have been observed in the digestate at this pilot plant (Lehtomäki & Björnsson, 2006) and was attributed to leaching of metals from the steel tanks.

3.4 Summary

The results of this study show that is very important to determine the correct values of silage characteristics when using silage as a substrate for the production of methane, to avoid overestimation of TS-based methane yields and also to enable reliable evaluation over time, as silage quality may deteriorate, thus reducing the maximum expected methane yields.

The summary of experiences from German energy crop digestion plants (FNR, 2010) is very useful as a basis for comparison with the present results. Long HRTs are common in energy crop digestion if little or no manure is added. The strategy in the present study was to reduce the HRT (50 days) without manure addition by adding micronutrients to obtain more efficient energy crop degradation, a strategy that has been investigated previously (Demirel & Scherer, 2011; Hinken et al., 2008; Lebuhn et al., 2008; Takashima et al., 2011). In addition, the macronutrients N, P and S were added, which has not been well studied in energy crop digestion. It should be noted that nutrient addition was not optimised, and it may be possible to reduce the amount of nutrients added while still maintaining the performance. When the TS_{corr} based OLR was increased to 4.1 $kg/m^3 \cdot d$, the efficiency of substrate degradation decreased. However, none of the process disturbances previously observed in energy-crop-based digestion were seen up to an OLR of 3.4 $kg/m^3 \cdot d$. It thus appears that the combined addition of macro- and micronutrients can have the same stabilising affect as manure in anaerobic digestion (Bruni et al., 2010). In addition, the viscosity problems previously observed in the mono-digestion of energy crops (FNR, 2010) were not seen here. The digester fluid had a viscoplastic and thixotropic behaviour but did not present stirring problems when the sludge was continuously mixed. Increased EPS concentrations were

observed at increasing load, but a correlation between EPS and viscosity could not be made in this study. Hence, more research is needed to establish the relationship between EPS and viscosity; and also to examine the effects of macronutrients addition on EPS secretion.

The effect of nutrient addition on the composition of the bio-fertilizer produced has been given little attention. Here it was shown that if macronutrients are not added, the digestate from degradation of this silage will not comply with the certification standards regarding heavy metals. The addition of Ni, which is one of the essential micronutrients in efficient methane production, will worsen the situation. In addition, the digestate will have very low N and P concentrations making it less attractive as a bio-fertilizer. Addition of N and P did not only contribute to the stability of the biogas process and good substrate degradation but also made the digestate more attractive as a bio-fertilizer. This could improve digestate utilization and provide potential economic benefit.

4. Conclusions

The present study has demonstrated that supplementation with macro- and micronutrients enabled stable and efficient biogas production for a process operating with energy crop mono-digestion (sugar beet/maize silage) with a 50-day HRT, up to an OLR of 3.4 $kg/m^3 \cdot d$. This was evidenced by good methane yields, low VFA concentrations and low residual methane production in the process. The addition of macronutrients also provided a digestate that complied with certification limits on heavy metal contents in bio-fertilizer for farmland application.

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Paper IV

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Benefits of supplementing an industrial waste anaerobic digester with energy crops for increased biogas production

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ABSTRACT

Currently, there is increasing competition for waste as feedstock for the growing number of biogas plants. This has led to fluctuation in feedstock supply and biogas plants being operated below maximum capacity. The feasibility of supplementing a protein/lipid-rich industrial waste (pig manure, slaughterhouse waste, food processing and poultry waste) mesophilic anaerobic digester with carbohydrate-rich energy crops (hemp, maize and triticale) was therefore studied in laboratory scale batch and continuous stirred tank reactors (CSTR) with a view to scale-up to a commercial biogas process. Co-digesting industrial waste and crops led to significant improvement in methane yield per ton of feedstock and carbon-to-nitrogen ratio as compared to digestion of the industrial waste alone. Biogas production from crops in combination with industrial waste also avoids the need for micronutrients normally required in crop digestion. The batch co-digestion methane yields were used to predict co-digestion methane yield in full scale operation. This was done based on the ratio of methane yields observed for laboratory batch and CSTR experiments compared to full scale CSTR digestion of industrial waste. The economy of crop-based biogas production is limited under Swedish conditions; therefore, adding crops to existing industrial waste digestion could be a viable alternative to ensure a constant/reliable supply of feedstock to the anaerobic digester.

1. Introduction

Currently, there is great interest in anaerobic digestion (AD) as a means of producing methane-rich biogas from the biological degradation of biomass available locally, such as industrial waste, agricultural waste, municipal solid wastes and wastewaters and, most recently, energy crops. For AD to be economically viable, a continuous supply of feedstock is a required (Lindorfer et al., 2008), which is not always possible in some regions due to increased demand for waste (Lebuhn et al., 2008). Consequently, there is a need for feedstock supplementation, in order to avoid fluctuations in feedstock availability (Lindorfer et al., 2008).

The type and composition of feedstock used in anaerobic digestion can greatly affect the stability, performance, and ultimately, the methane productivity of the process. Municipal and industrial waste, rich in lipids and proteins, are attractive as feedstock due to the high methane yields that can be obtained from these materials (Cirne et al., 2007; Hwu et al., 1998; Pereira et al., 2005). A mixed feedstock is also more likely to be well balanced in terms of the concentration of macro- and micronutrients. However, lipid degradation products (long-chain fatty acids) have been reported to severely inhibit methanogenesis (Luostarinen et al., 2009). Also, increasing free ammonia concentration that results from the degradation of proteins has been reported to be inhibitory to aceticlastic methanogens (Hansen et al., 1998; Schnürer and Nordberg, 2008).

Anaerobic digestion of energy crops is gaining ground. Energy crops are dedicated crops cultivated especially for energy production. They can be stored, through the process of ensiling, so that energy can be produced when the demand for, or price of, energy is high (Pakarinen et al., 2008). AD of energy crops alone has been plagued by process imbalance, a condition whereby the rate of feedstock hydrolysis and fermentation outweighs methane production through methanogenesis. Poor methane productivity has been reported as a result of low levels of macro- and micronutrients (Hinken et al., 2008; Pobeheim et al., 2010). Nutritional deficiencies, inappropriate amounts of macro- and micronutrients, and inadequate alkalinity may result in incomplete, unstable bioconversion of the feedstock, and may ultimately cause digester failure (Demirel and Scherer, 2008). For the AD process to be productive and sustainable, the concentration of macro- and micronutrients such as nitrogen (N), phosphorus, sulfur, iron, nickel, selenium, tungsten, cobalt, and molybdenum, must be in suitable range (Chen et al., 2008; Demirel and Scherer, 2008; Hinken et al., 2008). The ratio between C and N (C:N) in the feedstock is one of the parameters that have received most attention to date, and a C:N ratio of 16–20 has been

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suggested for stable AD processes (Álvarez et al., 2010; Mshandete et al., 2004). These conditions, and suitable contents of other macro- and micronutrients, can be achieved by the co-digestion of appropriate feedstocks.

Apart from improving the reliability of feedstock, co-digestion can offer other benefits, such as better cost efficiency, increased biodegradation, dilution of inhibitory compounds, improved nutrient balance, and increased biogas production (Mata-Alvarez et al., 2000; Misi and Forster, 2002; Murto et al., 2003; Sosnowski et al., 2003; Stroot et al., 2001). Some authors have shown that methane yield and process performance were improved significantly when energy crops were co-digested with manure (Cavinato et al., 2010; Comino et al., 2010), in contrast to the poor methane yields when crops were digested alone (Hinken et al., 2008; Pobeheim et al., 2010).

The aim of this study was to investigate the potential benefits of waste and energy crop co-digestion. The study was designed based on the operating conditions of a full-scale biogas digester in a region with high competition for waste suitable for AD. The full-scale biogas plant had a feedstock supply of industrial waste rich in proteins and lipids, varying considerably in amount and composition over the year. The amounts of energy crops required for full-scale operation were identified, and energy crops suitable for cultivation in the region were investigated. The main objective was to compare methane yields during operation with recurring lack of feedstock, to operation with energy crop supplementation. Also, the possibility of achieving a good balance between nutrients and carbon source in the feedstock was studied. Inoculum and industrial waste were collected at a full-scale plant, and hemp, maize and triticale (a hybrid of wheat and rye grown mostly for forage or fodder) were investigated as co-digestion feedstocks. Feedstock analyses as well as experimental data from both continuous and batch laboratory-scale experiments were used to evaluate the effect of waste and crop co-digestion.

2. Materials and methods

2.1. Inoculums and feedstock

The inoculum for the continuous laboratory-scale experiment was collected from the full-scale anaerobic digester used as reference in this study (Söderåsens Bioenergi, Sweden). The inoculum had a pH of 8.0, a partial alkalinity of 13.0 g/l, and a total alkalinity of 14.4 g/l. Other characteristics of the inoculum are given in Table 1. The effluent from the continuous laboratory-scale experiments was used as inoculum in the biochemical methane potential (BMP) tests.

The industrial waste feedstock was made up of pig manure, slaughterhouse waste, food processing and poultry wastes, and its composition varied throughout the year. The waste used for laboratory-scale experiments was collected on one occasion at the full-scale plant from the outflow after hygienisation (treatment at 70 °C for 1 h). The waste was filtered through a 1.6 mm sieve (Retsch GmbH, Germany) to separate the liquid slurry from the solids. Both fractions were retained as feedstock for the experiments. The industrial waste had a density of 1.01 ton/m^3. Other characteristics of the waste are given in Tables 1 and 2. The waste was frozen at −20 °C prior to thawing and use in the experiments.

Hemp, maize, and triticale were collected from energy crop cultivation trials (The Swedish Agricultural University, Alnarp, Sweden), where fertilization was performed with biogas plant effluent, and were used as co-feedstocks. Maize was harvested at full ripeness, and triticale was harvested at the early dough stage, based on recommendations by Amon et al. (2007a,b). Hemp was harvested in September based on recommendations by Kreuger et al. (2011b). Maize and triticale were chopped manually to about 5 mm pieces using a knife. Hemp was ground to pass through a 1.6 mm sieve (Retsch GmbH, Germany). The samples were thereafter stored frozen, fresh from harvest. Maize from the same harvest was also stored in full scale silage tubes for 7 months, prior to use in this study. The characteristics of all the feedstocks used in the study are listed in Table 1.

2.2. Experimental setup and operational protocol

2.2.1. Monitoring of the full-scale plant

The full-scale biogas plant (Söderåsens Bioenergi, Sweden) was a mesophilic, one-stage continuous stirred tank reactor (CSTR) with a working volume of 4200 m^3. The process was fed semicontinuously 10–12 times per day. The designed maximum daily addition was 220 m^3 with a maximum total solids (TS) content of 12%. The incoming waste was mixed in a reception tank, and thereafter passed through a 1-h batch hygienisation tank at 70 °C prior to feeding, (hygienisation is an EU prerequisite for waste of animal origin, EG 1774/2002). The material inflow and the raw gas volume were monitored online. The methane content of the gas and the TS in the waste mixture in the reception tank were monitored offline, once a day, Monday to Friday. The chemical composition of the feedstock was analyzed 23 times during the 1-year investigation period. These values were used to calculate the operational conditions in the full-scale digester for 12 months prior to the present study. Samples from the digester were also analyzed for pH and total ammoniacal nitrogen (TAN), from which the free ammonia was calculated.

Table 1
Characteristics of the materials used as inoculum and feedstock in the laboratory-scale experiments.

	Inoculum (CSTR)	Industrial waste	Maize	Hemp	Triticale
C:N ratio	N/A	10	34	24	44
% (w/w)					
TS	3.1	9.2	25.7	31.3	37.0
VS	1.9	8.0	24.2	28.8	35.4
mg per kg TS					
N	141,900	52,220	12,730	17,300	9930
P	15,260	7830	1840	3040	1500
S	14,500	3820	770	1310	580
Fe	27, 870	11,570	60	75	32
Ni	14.73	4.57	0.61	0.98	0.15
Mo	2.78	1.00	0.26	0.38	0.48
Co	3.50	1.37	0.05	0.07	0.03
W	0.25	0.12	0.01	0.01	0.06
Se	1.75	0.66	bdl	0.25	0.14

N/A not applicable and bdl below detection limit.

Table 2
Average composition (±1 SD) for the 23 samples of industrial waste feedstock removed/collected during 1 year of monitoring, and composition of the sample used for laboratory-scale investigations.

Sample	TS	VS	Ash	Crude fat	Protein	Carbohydrates
Average during the year	9.2 ± 1.9	8.0 ± 1.7	1.2 ± 0.3	3.5 ± 1.7	1.8 ± 0.8	2.8 ± 2.0
Laboratory-scale investigation	9.2	8.0	1.2	3.6	1.4	3

2.2.2. BMP tests

The potential methane production of the feedstocks was investigated in a BMP trial. The industrial waste, fresh maize, ensiled maize, hemp, and triticale were digested separately and in combination with the industrial waste (base feedstock). The basic experimental unit has been described previously by Nges and Liu (2009). All tests were performed in triplicate under the following operating conditions: 37 °C, mixing at 70 rpm, and 300 ml of inoculum with TS of 2.9% and volatile solids (VS) of 1.4%. The ratio of the inoculum VS to substrate VS was set at 2:1 in the assays, and the corresponding wet weight of each feedstock was calculated using the data given in Table 1. Mixtures of industrial waste and each energy crop, at a VS ratio of 1:1, were investigated in the co-digestion trials. This gave energy crop contributions of 25%, 22%, and 19% of the wet weight (WW) of the total feedstock for maize/ensiled maize, hemp, and triticale, respectively.

Control assays with only inoculum, and with both inoculum and microcrystalline cellulose (Avicel PH-101, Sigma–Aldrich, St. Louis, MO, USA) were also performed. Before starting/performing the analyses, anaerobic conditions were established by sparging with nitrogen gas prior to corking of the flasks. During the experiments, gas composition and total gas volume were monitored every other day. TAN, volatile fatty acids (VFAs), and pH were determined at the end of the experiment. The experiments were terminated after 31 days of incubation when the methane production rate in all assays had decreased below 2 m^3 CH_4/ton WW per day.

2.2.3. Continuous laboratory experiments

The laboratory experimental setup consisted of six 3-l, jacketed glass CSTRs and three 1-l, 4 °C cooled substrate vessels. The biogas produced was collected through gas-tight Tygon® tubing (Saint-Gobain PA, USA) in air-tight gas collection balloons (Transofoil, Flextrus, Lund, Sweden). The reactors were initially inoculated with seed sludge from the full-scale process. Impellers (EURO-ST D, Germany) rotating at 80 rpm were used to mix the reactor contents and substrates. The working volume of the reactors was 2.5 l, and they were maintained at 37 °C by circulating hot water through the water jacket (Newington, USA). The filtered fraction of the industrial waste was fed to the reactors from the 4 °C cooled substrate vessel 10 times per day (75 g per day in total) with the aid of piston pumps. The solid fractions (3.5 g) were fed manually with a home made-100 ml plastic syringe, four times per week, through a port on the side of the reactor. The total amount of feedstock added per day (solid and liquid fractions) was 77 g, corresponding to an organic loading rate (OLR) of 2.5 gVS/(l.d) and a solid retention time (SRT) of 32.5 days. These conditions were maintained in all six reactors for a period of 100 days. The initial plan was to add energy crop in four of the CSTR replicates after this period. This was however not the case due to the high standard deviations during the initial 100 days despite all procedural improvements. These high standard deviations would make any difference in methane yields with and without crop addition not statistically significant. The only results given from the CSTR experiments are thus the methane yields from mono-digestion of the industrial waste.

2.3. Analytical methods

TS, VS, and pH were determined according to standard methods (APHA, 1995). For the maize silage, TS and VS determination were not based on oven drying, due to the errors that may arise because of the presence of volatile compounds in the silage (Kreuger et al., 2011a; Mukengele and Oechsner, 2007). Maize silage methane yields were thus based on WW only. Biogas composition was determined by gas chromatography, as described elsewhere (Parawira et al., 2004). The compounds detected were methane, carbon dioxide, oxygen, and nitrogen. The total gas volume was measured using a graduated 100-ml gas-tight glass syringe with a sample lock (Fortuna, Germany) in the batch experiments, and a wet-type gas meter (Schlumberger, Karlsruhe, Germany) for the continuous experiments. Methane and biogas yields were calculated as the net amount of methane produced per unit WW or VS added to the digester, normalized to a temperature of 0 °C and assuming a constant pressure of 1 atm. The lower heating value of methane, 9.97 kWh/m^3 was used for energy calculations.

VFAs were analyzed with HPLC (Varian Star 9000, Varian, Walnut Creek, CA, USA), with a Biorad column, Cat. 125-0115 (Hercules, CA, USA) as described previously by Parawira et al. (2004). The TAN concentrations were measured with the Dr. Lange LCK 303 analytical kit (Dr. Bruno Lange GmbH, Dusseldorf, Germany) after diluting a 0.45 μm filtered sample to fall within the detection range. The non-ionized (free ammonia, FA) fraction of the TAN was calculated as described elsewhere (Angelidaki and Ahring, 1993). Samples were weighed using an electrically powered 3-digit precision balance (Sartorious excellence, Göttingen GmbH, Germany). Alkalinity was evaluated as partial alkalinity and total alkalinity, as described by Nges and Liu (2009).

The industrial waste was analyzed at Eurofins Food & Agro Sweden AB (Linköping, Sweden) to determine crude fat and protein content. All VS that were not fat or proteins were assumed to be carbohydrates. The concentrations of macro- and micronutrients were analyzed in the industrial waste and the fresh crops by LMI AB (Helsingborg, Sweden), using elementary analysis for the N content and the C:N ratio, ICP-OES for S, P, and Fe, and ICP-MS for Ni, Mo, Se, W, and Co.

2.4. Statistical analysis

Grubb's test as used to insure there were no outliers in the batch test replicates, and a *t*-test was performed to compare the mean values obtained in the batch experiments. *t*-Test was also used to compare the recorded and expected methane yields in the co-digestion experiments. Comparisons rendering a *p*-value smaller than 0.05 were considered to be statistically significant. Turkey's multiple comparison test (one-way ANOVA) was used to compare replicates in the laboratory CSTR experiments.

3. Results and discussion

3.1. Full-scale operation

Fig. 1 shows the daily full-scale feedstock addition over a 1-year period (July 2007 to June 2008), which averaged 155 ± 32 m^3 industrial waste per day, with an average TS of 9.2 ± 1.8%. During this period, 23 samples of the full-scale feedstock (industrial waste) were collected for compositional analysis. The average sample composition is given in Table 2. The average OLR in the full-scale process was 3.2 ton VS/(m^3 .d) with an HRT of 27 d.

The pH in the digester averaged 8, while the TAN averaged 4 g/l, giving an FA concentration of 375 mg/l. FA concentrations above 128–330 mg/l have been shown to cause a shift in the methane production pathway to involve syntrophic acetate oxidation (Schnürer and Nordberg, 2008). This pathway progresses slower than aceticlastic methanogenesis, which increases the risk of VFA accumulation and process instability. The inhibitory effects of FA have previously been reported in the AD of protein-rich feedstock and manure (Angelidaki and Ahring, 1993; Chen et al., 2008; Hansen et al., 1998; Kapp, 1992).

The average methane yield was 46 ± 9 m^3 CH_4 per ton feedstock. Using the average VS for the 23 analyzed samples to calculate the VS-based yields gives 575 m^3 CH_4 per ton VS. Similar yields have been reported for the anaerobic digestion of lipid- and protein-rich substrates (Luostarinen et al., 2009).

The process was periodically under-utilized, as the full capacity of the plant was 220 m^3 feedstock with 12% TS per day (Fig. 1). The already high FA concentration in the process indicates that only a co-feedstock with less nitrogen was suitable. Therefore, co-digestion of the industrial waste with carbohydrate-rich energy crops is a plausible alternative.

The industrial waste used as feedstock in the laboratory-scale experiments was collected at the end of the full-scale monitoring period. It had a crude fat and protein content similar to that of the average feedstock composition over the year (Table 2).

3.2. Feedstock characterization

The composition of the industrial waste and the nutrient content in the feedstocks used in the study are given in Tables 1 and 2. Industrial waste had a high fat content and a C:N ratio of 10. It also had a higher amount of all the macro- and micronutrients investigated, and a very high content of N, P, and Fe as compared to the crop samples. The crops, on the other hand, were poor in essential micronutrients, with C:N ratios ranging from 24 to 44. Combining industrial waste and crops in the laboratory co-digestion experiments such that the crops contributed 19–25% of the total feedstock improved the C:N ratio to values between 15 and 17. The optimal C:N ratio for anaerobic digestion has been reported to be between 16 and 20 (Álvarez et al., 2010; Mshandete et al., 2004). On the other hand, as have been reported in other studies, digestion of the energy crops alone would probably not be successful as they contained low amounts of essential macro- and micronutrients such as N, P, S, Ni, Co, and Mo (Demirel and Scherer, 2008; Hinken et al., 2008; Pobeheim et al., 2010). In a biogas plant treating industrial waste together with energy crops, the waste will contribute nitrogen and the essential nutrients, while the crops will contribute carbohydrates. Neves et al. (2008) have reported better process performance when the feedstock contains equivalent amounts of fats, proteins and carbohydrates.

3.3. Methane production from batch mono- and co-digestion

The results of the batch mono- and co-digestion experiments are shown in Fig. 2. All feedstocks, except hemp alone, had produced 90% of the final methane yield after 16 days of digestion in mono- and co-digestion. The industrial waste digested alone gave a methane yield of 59.3 ± 2.2 m^3 CH_4/ton feedstock, corresponding to 723 ± 26 m^3 CH_4/ton VS added. The relatively low yield per ton of feedstock is due to the high water content of the material, but the high methane yield per unit organic matter is due to the excellent biodegradability of the industrial waste and its chemical composition (being rich in lipids and proteins). The yield corresponds well to theoretical yields calculated based on the chemical composition of the industrial waste (Davidsson, 2007). Similar results have been reported by Cirne et al. (2007) in batch anaerobic digestion of lipid-rich waste.

Mono-digestion of energy crops gave methane yields in the range expected for carbohydrate-rich crops. The yields (m^3 CH_4/ton WW) ranged from 75 to 140 (Fig. 2), corresponding to methane yields ranging from 260 to 396 m^3 CH_4/ton VS added. Other authors have reported yields in the same range; e.g. Amon et al. (2007a,b) reported 398 ± 23 m^3 CH_4/ton VS added for different maize varieties, and 312–365 m^3 CH_4/ton VS added for maize at full ripeness. Maize silage gave a methane yield of 97.1 ± 5 m^3 CH_4/ton WW added, as opposed to 89.7 ± 9 m^3 CH_4/ton WW added for fresh frozen maize. Comparison of the mean values using a one-sided *t*-test showed no significant difference in the yields of fresh frozen and ensiled maize. Similar findings have been reported by Mukengele and Oechsner (2007). Hemp digestion resulted in the lowest yield per ton of VS (262 m^3) amongst the crops. This can be attributed to the presence of inhibitory compounds such as alkaloids, or the lignocellulosic fibrous structure of hemp as reported by Kortekaas (1995) and Kreuger et al. (2011b). Kreuger et al. (2011b) have also reported methane yields between 227 and 249 m^3 CH_4/ton VS from the thermophilic digestion of hemp harvested at different times of the year.

Co-digestion led to significant improvements in WW-based methane yields, up to 32%, compared to the industrial waste alone (Fig. 2). The dry nature of the crop co-feedstock meant that more methane was produced from the mixture of feedstocks, than the industrial waste alone, which has good biodegradability but lower TS. The high nutrients content in the industrial waste co-feedstock (Table 1) may have facilitated the digestion of the poor nutrient crop co-feedstock (Table 1) as was seen by the improved biodegradation of the co-digestion mixtures. Previous studies have attributed the increased methane yield in co-digestion to two factors: (i) direct methane contribution from the co-feedstock, and (ii) a synergetic effect due to the complementary characteristics of the two feedstocks, where a better nutrient balance leads to improved biodegradation (Chen et al., 2010; Comino et al., 2010;

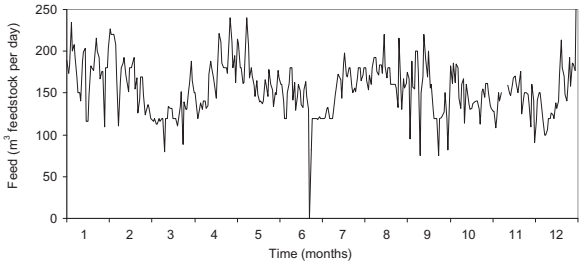

Fig. 1. Feeding regime (m^3 feedstock per day) in full-scale operation during a 1-year period.

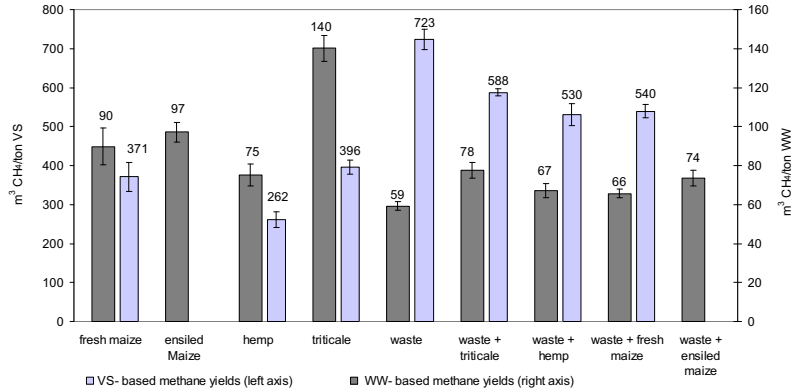

Fig. 2. Methane yields (N m^3 CH_4/ton VS and N m^3 CH_4/ton WW) for batch mono- and co-digestion of industrial waste and energy crops. The bars indicate one standard deviation of the replicates.

Parawira et al., 2004). These studies reported significant improvements in methane yield and process stability when different feedstocks such as potato and beet leaves (Parawira et al., 2004), vermicompost and cornstalk (Chen et al., 2010), and maize silage and manure (Comino et al., 2010), were co-digested compared to digestion of an individual feedstock.

The recorded or experimented co-digestion methane yields weighted per ton VS (Fig. 2) were not significantly different from the expected or calculated methane yields (half the sum of methane yields from the industrial waste and the crop co-feedstock). Experimented and expected co-digestion methane yields based on WW also did not differ significantly. This showed that co-digestion did not lead to any adverse effect. Though the industrial waste was richer in nutrients as compared to the crops (Table 1), a condition which should upset any nutritional deficiencies in the co-digestion feedstock, mono-digestion of the feedstocks also showed good biodegradation probably as results of the nutrients from the inoculum.

The findings in this study demonstrated that batch anaerobic with a nutrient-rich inoculum (Table 1) and the 2:1 inoculum to feedstock ratio resulted in an excellent biodegradation of all the feedstock in the mono- and co-digestion experiments.

In summary, crop digestion resulted in higher methane yields per ton of feedstock, than the industrial waste alone, as a result of the high dry matter content of the energy crops, while industrial waste showed high methane yields per ton of organic matter due to better degradability. Good and rapid biodegradation facilitated by macro and micro-nutrients from the industrial waste and or inoculum was noted for all feedstocks apart from fiber-rich hemp alone.

The pH at the end of the batch experiments ranged from 7.7 to 8.1, and TAN ranged from 2990 to 3570 mg/l. The calculated FA concentration after digestion ranged from 357 mg/l for the industrial waste to 270 mg/l for hemp. Co-digestion of industrial waste and hemp gave an FA concentration of 305 mg/l. This shows that co-digestion of crops with industrial waste could reduce the FA concentration, even in a heavily inoculated, nitrogen-rich process.

3.4. Laboratory-scale CSTR

The six replicate reactors in the continuous laboratory-scale experiments were maintained at an OLR of 2.5 g VS/(l days) and a 32.5-day SRT to mimic periods of lower feedstock addition in the full-scale plant. These conditions were maintained for 100 days by feeding with industrial waste alone. During the initial 100 days, the methane yield was 55 ± 11 m^3 CH_4/ton feedstock, or 689 ± 141 m^3 CH_4/ton VS. The high standard deviation of these results was due to clogging of feeding tubes by the cooled lipid-rich feedstock; in fact the cooling of the substrate vessels caused the fat-rich industrial waste to coagulate and form lumps. These lumps of fat led to an uneven load in the reactors, both in terms of substrate composition, and causing blockages in the feed pumps. This high deviation would have made it impossible to continue as planned and evaluate the effect of crop addition on methane yield, since no differences would have been likely to be statistically significant. Only the value obtained from the digestion of industrial waste alone is used in further calculations and discussions. Digestion of the industrial waste resulted in a relatively high pH (7.8–8.1). The average TAN concentration was 3.8 g/l, giving an average FA concentration of 380 mg/l calculated with the formula reported in Angelidaki and Ahring (1993). This FA concentration is in the same range as in the full-scale plant.

3.5. Laboratory-scale results and full scale operation

The methane yields for the industrial waste were 46 ± 9, 55 ± 11, and 59 ± 2 m^3 CH_4/ton feedstock in full-scale CSTR experiments, laboratory-scale CSTR experiments, and laboratory-scale batch digestion experiments, respectively. High methane yields should be expected in batch experiments due to the excess of inoculum which, apart from an active microflora, contains essential nutrients and buffering capacity (Chen et al., 2008). The results from batch digestion studies can normally not be used for the prediction of full-scale yields from continuous trials. Here, however, a full-scale methane yield is available for comparison, operating on a substrate very similar to that of the laboratory scale study (Table 2). The full scale process gave on average 78% of the methane yield in batch trials for the industrial waste during a year period. This value was used as a conversion factor for the energy crops, to calculate the effect of crop addition in the full-scale plant. Crop addition based on a 1:1 WW ratio of maize and triticale was used in the calculations. The average TS (31%), C:N ratio (39) and methane yield (115 m^3/ton WW) for this mixture of crops was calculated based on the results given in Table 1 and Fig. 2. The methane yield was then reduced to 78% of the above value, giving 90 m^3/ton WW.

Table 3
Calculated full-scale characteristics and production increase resulting from the co-digestion of industrial waste and energy crops.

	Present full-scale operation	Adding crops to 12% TS	Adding crops to 220 ton/days
Waste addition (ton/days)	155	155	155
Crop addition (ton/days)	–	23	65
TS of feedstock (%)	9.2	12	16
C:N ratio	10	14	19
Methane yield ($N m^3$/ton WW)	46	52	59
Annual methane production (GWh)	26	33	47

Table 3 gives the conditions for full-scale operation. In addition, calculations were performed for two cases based on the potential crop addition to the full-scale plant:

(1) the addition of energy crops to reach the maximum average TS of 12% in the reception tank, still allowing the feeding of a pumpable feedstock, and
(2) addition of energy crops to reach the maximum design load of 220 ton feedstock addition per day.

Limiting the TS of the full-scale feedstock to 12% would give an annual addition of 8400 ton WW of crops, leading to an additional methane production of 7 GWh/year. Adding crops to utilize the full feeding capacity of 220 ton feedstock per day, 23,700 ton WW of crops per year, would lead to an additional methane production of 21 GWh/year, almost doubling the methane production of the plant when operating on industrial waste alone. However, this would result in TS of 16% for the mixture, which would be too dry for the present feed system (Table 3). Other drawbacks that could be encountered when adding energy crops to a system/plant not originally designed for co-digestion may be scum formation and fiber floatation, leading to poor process performance as a result of wash-out of biomass. Crop addition will however, help utilize the full capacity of the biogas plant. The methane yield per ton of feedstock will be increased in both cases, and by crop addition the yearly methane production could be designed to meet the full capacity of the plant and the gas upgrading system.

Based on the results of the analyses of the micro- and macronutrients, co-digestion of industrial waste with energy crops appears attractive. Anaerobic digestion of the industrial waste studied here is feasible on a large scale. However, the nitrogen content in the waste is so high that the FA concentration in the digester is inhibitory to aceticlastic *Archaea*. Process instability due to FA often results in VFA accumulation, and interactions between FA, VFAs, and pH may lead to an "inhibited steady state", a condition in which the process is stable but the methane yield is lower (Angelidaki and Ahring, 1993; Chen et al., 2008). The high nitrogen content of the industrial waste can be balanced by crop addition, while the energy crops can be converted into methane without the addition of external micronutrients. This finding is thus promising in the light of previous reports that anaerobic digestion of energy crops alone gives low methane yields without the addition of macro- and micronutrients (Hinken et al., 2008; Pobeheim et al., 2010). Cecchi et al. (1996) reported that the benefits of co-digestion include the use of existing infrastructure, an improved balance of nutrients, and an increased organic load of biodegradable feedstock. Finally, since periods of low supply of industrial waste recur in the full-scale plant, co-digestion with energy crops is suggested as a means of utilizing the full capacity of the plant.

4. Conclusions

In the present study, industrial waste and energy crops were successfully co-digested, resulting in an improved C:N ratio and reduced concentration of FA. This could in turn lead to better process performance and stability in a full scale operation. Co-digestion of energy crops with the industrial waste studied here would remove the need for micronutrients normally required in crop digestion. In addition, higher methane yields per ton of feedstock were achieved when the industrial waste was mixed with energy crops. Such findings have important implications. Expansion of AD processes has resulted in a high demand for waste, thus leading to competition for waste; hence new means must be explored to ensure a steady supply of feedstock. In this study, specifically grown energy crops were used for this purpose. Under Swedish conditions, however, the economy in crop-based biogas production is limited; therefore, adding energy crops to existing industrial waste digestion plants may be a viable alternative. In a situation where the supply of waste may be limited, this could improve the economy of the plant. For the specific full-scale plant used as a reference in this study, crop addition would also improve the nitrogen balance in the digester.

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Paper V

Improved utilization of fish waste by anaerobic digestion following omega-3 fatty acids extraction

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Abstract

Fish waste is a potentially valuable resource from which high-value products. Anaerobic digestion of the original fish waste and the fish sludge remaining after enzymatic pre-treatment to extract fish oil and fish protein hydrolysate was evaluated regarding the potential for methane production. The results showed high biodegradability of both fish sludge and fish waste, giving specific methane yields of 742 and 828 m^3 CH_4/tons VS added, respectively. However, chemical analysis showed high concentrations of light metals which, together with high fat and protein contents, could be inhibitory to methanogenic bacteria. The feasibility of co-digesting the fish sludge with a carbohydrate-rich residue from crop production was thus demonstrated, and a full-scale process outlined for converting odorous fish waste to useful products.

Keywords: salmon waste, biogas, Jerusalem artichoke

1. Introduction

In 2005, the UN Food and Agricultural Organisation has estimated the annual world fish harvest resulting from commercial fishing in wild

fisheries and fish farms to be 140 million tons (FAO, 2005). At ton in the present represent 1000 kg of weight. Fish processing generates considerable amounts of waste in the form of edible and non-edible by-products. Assuming 45% of the live weight to be waste (Rai et al., 2010), it can be estimated that nearly 64 million tonnes of fish waste are generated annually. This waste is mainly composed of heads, viscera, bones and scales, and is rich in lipids and proteins. Fish waste is often under-utilized (Berge, 2007) being mainly used in the production of low-value animal feed products such as fish meal or fish silage (Crexi et al., 2009; Liaset and Espe, 2008). Arvanitoyannis and Kassaveti, (2008) reported that highly valuable compounds such as fish oils, biodiesel, enzymes, omega-3 fatty acids and proteins can be obtained from fish waste. A good number of studies has been done on the extraction and purification of omega-3 polyunsaturated fatty acids (PUFAs) from fish waste (Liaset and Espe, 2008; Linder et al., 2005; Mbatia et al., 2010b). PUFAs are mainly present in marine oils and are associated with several health benefits (Mozaffarian et al., 2010; Patel et al., 2010). Extraction of oils is also important as oxidation of the unsaturated fatty acids present in fish oil is the major factor responsible for the offensive odour associated with fish and fish waste (Rai et al., 2010). The potential of using the soluble proteins or fish protein hydrolysate (FPH) for microbiological growth media has also been reported (Aspmo et al., 2005; Klompong et al., 2009). The extraction of PUFAs and FPH from fish waste will, however, result in a waste product that must be properly handled.

In 2006, Salmon constituted just over a million tons of the world's fish harvest (Gebauer and Eikebrokk, 2006). In a typical automated filleting line, the fillets account for approximately 59-63% of the total wet weight of a salmon weighing 5-6 kg (Liaset and Espe, 2008), hence about half a

million tons of salmon waste is generated annually. Our group is engaged in studying Salmon (*Salmo salar*) heads as they represent oil-rich fish waste. Oils have previously been extracted and PUFAs enriched by enzymatic hydrolysis in processes described by Mbatia et al., (2010a and 2010b). The extraction method applied also allows the straightforward removal of FPH, which was used as an additive to *Lactobacillus sp* growth media. The residual product from PUFAs and FPH extraction called fish sludge can be a substrate for biogas production through anaerobic digestion.

Anaerobic digestion has been used for waste treatment and biogas recovery from many types of organic waste. Its numerous advantages, such as the recovery of a renewable energy carrier, waste volume reduction and odour reduction are well documented (Parawira et al., 2008; Wu et al., 2009). Plant nutrients such as nitrogen and phosphorus are retained in the effluent (digestate) after anaerobic digestion, which can be used as a bio-fertilizer in agricultural production provided it meets the required standards. The heavy-metal content in digestate is regulated by different certification schemes in different countries as SPCR in Sweden (Berglund, 2010; SP, 2010). Legislation regarding the handling of animal by-products may also be applicable, which in the EU can involve heat treatment with a minimal particle size of 12 mm at 70°C for 1 hour (European Commission, 2006).

Waste such as fish waste and fish sludge, which are rich in lipids and proteins, have the advantage of giving high methane yields, and can be attractive as substrates in an anaerobic digestion process (Cirne et al., 2007). At the same time, these types of waste also have properties that make them less suitable for anaerobic microbial degradation, for example;

- Free long-chain fatty acids (LCFAs) can inhibit methanogenesis (Cirne et al., 2007; Pereira et al., 2005).
- Protein degradation causes high concentrations of free ammonia (NH_3) in the process, which might inhibit aceticlastic methanogenesis (Schnurer and Nordberg, 2008).
- High concentrations of light metals such as calcium, sodium, potassium and magnesium are known to be inhibitory to methanogens as has been reviewed by Chen et al. (2008).

Anaerobic digestion of protein-rich substrates such as meat and bone has been reported (Wu et al., 2009). A few studies have been reported biogas production from fish-related waste, such as sludge from saline fish farming and sludge from salmon farming (Arvanitoyannis and Kassaveti, 2008; Gebauer, 2004; Gebauer and Eikebrokk, 2006). Poor methane yields in anaerobic digestion of fish residues have been attributed to the inhibitory effects of lipids and ammonia, and co-digestion was investigated as means to overcome the inhibition (Gumisiriza et al., 2009; Mshandete et al., 2004).

In this study, the rest product from PUFAs and FPH extraction called the fish sludge was used for biogas production through anaerobic digestion. The possibility of biogas production from the original fish waste was also investigated for comparison. The prospect of co-digesting fish sludge with biomass containing a low content of the above compounds (Jerusalem artichoke) was investigated, as a strategy to avoid inhibition by NH_3, LCFAs and light-metal ions. Biochemical methane potential (BMP) assays, chemical analyses and calculations based on the experimental data were used to evaluate the feasibility of biogas production. The overall aim was to explore the feasibility of a bio-refinery approach, extracting multiple

products from waste, taking advantage of the differences in biomass components and intermediates, while limiting waste production.

2. Materials and methods

2.1 Substrates and inoculum

2.1.1 Fish waste and fish sludge

The salmon heads were minced and homogenised with a grinder (GM 200, Retsch GmbH, Germany). This fraction, called fish waste, was investigated with regard to biogas production without further treatment.

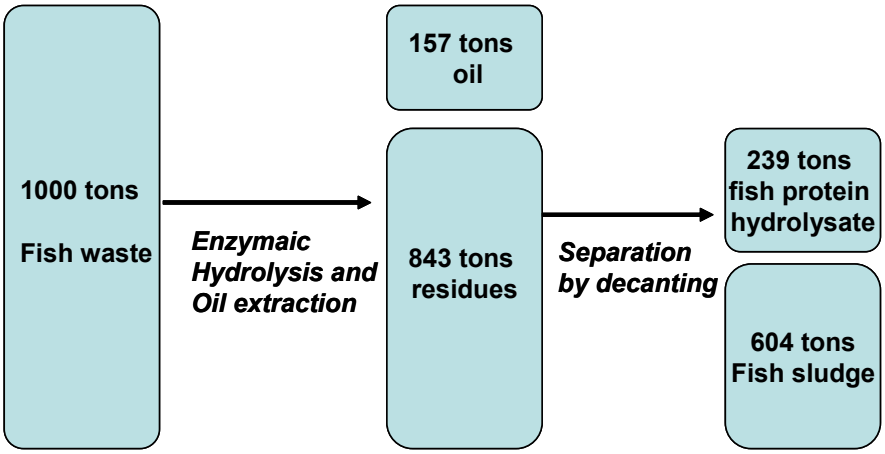

Figure 1 The products recovered, and fish sludge (FS) generated in the processing of 1000 tons of salmon waste. FPH stands for fish protein hydrolysate, FW stands for fish waste and 'waste' is the intermediate residue after oil extraction.

As reported in a previous study (Mbatia et al., 2010b), the crushed salmon heads were heated at 55 ^{o}C for 1 h, enzymatically hydrolysed with the aid of a commercial proteases (bromelain and Protex 30L) and centrifuged at

2000 x g to extract the oil and separate (by decanting) the aqueous protein-rich phase (FPH) from the fish waste. Best oil yield (15.7 g from 100 g of fish waste) was obtained when the fish waste was hydrolysed using 0.05% (v/w) Protex 30L without pH adjustment or water addition

The residue from this extraction and separation, called fish sludge, was also investigated with regard to biogas production. The amounts of the products derived from 1000 tons of salmon waste (0.2% of the annual global salmon waste produced) are illustrated in Figure 1.

2.1.2 Crop biomass

The above-ground part of Jerusalem artichoke (*Helianthus tuberosus*) was chosen to represent a residual biomass from crop cultivation, as it is typically high in carbohydrates but low in fats, protein and salts. The plant is native to North America, but grows well in many climates. The tuber is used as a vegetable, while the above-ground biomass is considered a residue. The Jerusalem artichoke used in this study was cultivated in southern Sweden (55 40′N 13 6′E) and harvested in October. The leaves and stems were chopped into about 2 cm pieces with a garden shredder (AXT 2500 HT, Robert Bosch GmbH, Germany). They were then minced in the laboratory with the GM 200 grinder so as to pass through a 6 mm mesh.

2.1.3 Inoculum

The inoculum used for anaerobic digestion was the effluent from a full-scale biogas plant (Söderåsens Bioenergi, Sweden). This biogas plant treats food industry waste from different sources and normally operates under high concentrations of ammonia nitrogen (NH_4-N). The inoculum was also rich in both macro- and micronutrients as has been reported in a previous

study (Nges et al., 2012). The buffering capacity (partial alkalinity) was 5.9 g/L, the NH_4-N was 4.0 g/L and the pH was 8.

2.2 Biochemical methane potential assay

The biochemical methane potential (BMP) was determined using the method described by (Kreuger, 2011), but at 37°C, with 300 mL of inoculum per assay, crystalline cellulose as the control (Avicel PH-101, Sigma-Aldrich, St. Louis, MO, USA) and no nutrients were added to the assays. During the experiments, the gas composition and total gas volume were monitored every other day. Ammoniacal nitrogen (NH_4-N) and pH were determined at the end of each experiment. The experiments were terminated after 33 days of incubation.

Fish waste, fish sludge and Jerusalem artichoke were digested separately in mono-digestion BMP assays. The fish sludge was co-digested with Jerusalem artichoke at ratios of 1:1 and 1:3 based on the content of organic material measured as volatile solids (VS). All experiments were performed in triplicate. The gas volumes are given as dry gas normalized to standard temperature and pressure (0°C, 101.3 kPa), and the methane yield is reported as the *normalized* volume of methane divided by the amount of VS added of each substrate or mixture of substrates.

2.3 Analytical methods

Total solids (TS), VS and pH were determined according to standard methods (APHA, 1995). Total nitrogen, macro minerals and light metals (sulphur (S), phosphorous (P), potassium (K), sodium (Na), calcium (Ca), magnesium (Mg)) and heavy metals (cadmium (Cd), lead (Pb), zinc (Zn), nickel (Ni), chromium (Cr), copper (Cu) and mercury (Hg)) were analysed

by LMI AB (Helsingborg, Sweden) using the Kjeldahl method, inductively coupled plasma–optical emission spectrometry (ICP-OES) and inductively coupled plasma–mass spectrometry (ICP-MS), respectively. The NH_4-N concentrations were measured with the LCK 303 Dr. Lange test kit (Dr. Bruno Lange GmbH, Düsseldorf, Germany) after diluting a 0.45 μm filtered sample to fall within the detection range. Methane content in biogas was determined by gas chromatography with a thermal conductivity detector as earlier reported elsewhere (Parawira et al., 2008). The total gas volume was measured using a graduated 100-mL gas-tight glass syringe with a sample lock (Fortuna, Germany).

2.4 Calculations

The non-ionised fraction of the NH_4-N, NH_3 was calculated as described elsewhere (Angelidaki and Ahring, 1993). Based on the experimentally determined methane yields and the chemical analyses, calculations were made to reproduce the potential full-scale conditions in an anaerobic co-digestion process. The mass loss and VS reduction during digestion were calculated by subtracting the total mass of CH_4 and CO_2 formed, quantified using the experimentally determined methane yields. The potential loss of other compounds, e.g. N_2, H_2 H_2S, NH_3 etc., through the raw gas was assumed to be negligible, and these were not included in the calculations. This means that all minerals in the ingoing substrate remained in the effluent, i.e., the bio-fertilizer. The only change assumed was that organically bound nitrogen was partly mineralized. The degree of mineralization of organically bound nitrogen was assumed to be equal to the degree of VS degradation. The lower heating value (9.97 kWh/m^3) was used to convert normalized methane volume into units of energy.

2.5 Statistical analyses

A significance test (one-way ANOVA) was performed to verify whether co-digestion led to any significant difference in methane yield. Grubb's test was used to ensure there were no outliers in the test replicates, and a t-Test was performed to compare the means in the experiments. Statistical significance was defined as $p \leq 0.05$.

3. Results and discussion

3.1 Material flow and chemical analyses

The amount of residual fish sludge after PUFA and FPH extraction and separation from fish waste is illustrated in Figure 1. The fish sludge constituted about 60% after the removal of oil and FPH. The components of the investigated materials and the inoculum are listed in Table 1.

The fish sludge contained 52% and 49% of the TS and VS of the original fish waste respectively, hence the amount of waste remaining after the extraction of oil and FPH was significant. Table 1 shows the very high nitrogen concentration of both fish sludge and fish waste, compared to that of the crop residue (Jerusalem artichoke) and the concentration in the effluent from a large-scale biogas process (inoculum). This shows that it is not feasible to digest either fish waste or fish sludge alone in a biogas process due to the very high ammonia nitrogen concentrations. The concentrations of light metals are also high, while heavy metals (with the exception of zinc), which could jeopardize the bio-fertilizer quality, are not higher than in the investigated crop sample and inoculum.

Table 1 Composition of substrates and inoculum used in the anaerobic degradation trials

Variables	Inoculum	Fish waste	Fish sludge	Jerusalem artichoke
TS (%)	3.7	41.2	37.7	24.7
VS (%)	2.0	35.5	31.4	21.7
Concentrations (mg/kg wet weight)				
N	8010	23800	26100	5340
P	440	7700	8700	380
S	360	1800	1900	280
K	1200	1600	1500	1430
Na	1500	1800	1800	64
Ca	760	11000	13000	6170
Mg	66	380	410	1480
Cd	0.02	0.01	0.03	0.09
Pb	0.3	0.3	0.2	0.5
Zn	8.6	88	71	4.8
Ni	0.50	0.06	0.17	0.23
Cr	0.49	0.29	0.25	0.15
Cu	4.00	0.90	0.91	1.24
Hg	<0.01	0.02	0.03	0.01

3.2 Biochemical methane potential

3.2.1 Mono- digestion of fish waste, fish sludge and Jerusalem artichoke

The extent of conversion of the various substrates, in terms of methane yield, is shown in Figure 2 (together with the results of co-digestion experimented and calculated). Jerusalem artichoke showed the lowest methane yield of the feedstocks investigated (283 ± 14 m^3CH_4/ton VS added, corresponding to 61 ± 3 m^3CH_4/ton wet weight, WW, added). This result is in concordance with earlier report by Gunnarson et al. (1985) where a methane yield of 315 m^3CH_4/ton VS added was achieved in the

anaerobic digestion of the above-ground part of Jerusalem artichoke. The cellulose control used in the BMP assay rapidly reached the theoretical methane yield, showing that the inoculum had cellulolytic activity for crystalline cellulose (results not shown). The low methane yield for Jerusalem artichoke leaves can be attributed to recalcitrant compounds such as the lignin embedded cellulose in plant fibres (Amon et al., 2007).

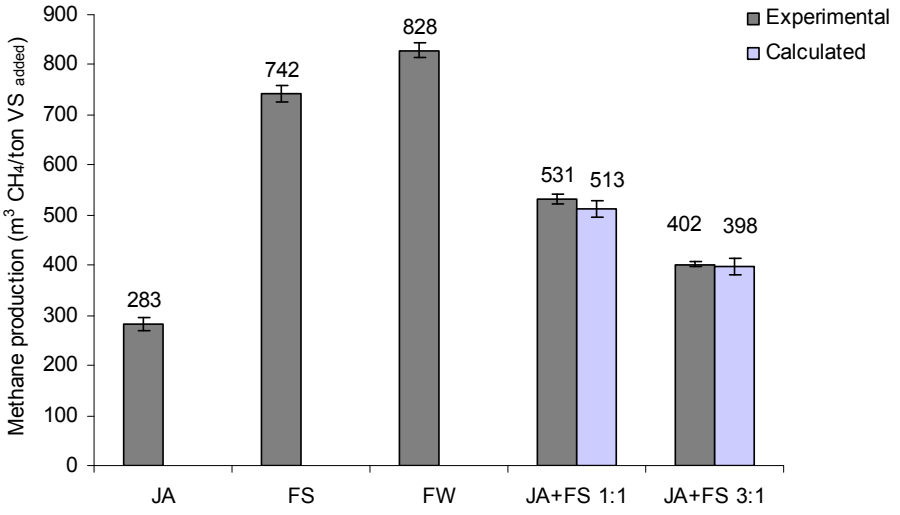

Figure 2 Methane yields from the separate digestion of Jerusalem artichoke residues (JA), fish waste (FW), fish sludge (FS,) and co-digestion of mixtures of FS and JA at VS ratios of 1:1 and 1:3. The calculated values are also given

The anaerobic digestion of fish waste gave a methane yield of 828 ± 15 m^3CH_4/ton VS added, corresponding to 294 ± 6 m^3CH_4/ton WW added, while fish sludge gave 742 ± 17 m^3CH_4/ton VS added, corresponding 234

± 5 m^3CH_4/ton WW added. Statistical analysis of the different between fish waste and fish sludge showed that there was a significant difference in methane yield ($p \leq 0.05$). The difference can be explained by the removal of oils and proteins from the fish waste, but the high yield of fish sludge also shows that some residual oil remained in this sample. The enzymatic oil extraction method used in generating fish sludge in this study is known to give lower oil yields than solvent extraction (Mbatia et al., 2010b). Fish waste and fish sludge can be expected to have very high methane yields due to their high lipid content and proteinous nature. The theoretical yield for lipids is about 1000 m^3CH_4/ton VS, while the theoretical yield for protein is about 490 m^3CH_4/ton VS (Moller et al., 2004). In addition, the BMP assays were highly inoculated thereby eliminating any risk of inhibition from compounds such as NH_3, LCFAs and light metal ions.

In other studies on the anaerobic digestion of fish residues, low methane yields resulting from inhibition have been reported. Gebauer and Eikebrokk, (2006) reported methane yields in the range of 260-280 m^3CH_4/ton VS added in the mesophilic treatment of salmon sludge (generated from salmon farming).The low yields were attributed to high concentrations of volatile fatty acids and ammonia, a condition reported elsewhere as inhibited steady-state (Angelidaki and Ahring, 1993). Poor methane yields have also been reported by Gebauer, (2004) as being due to high concentrations of sodium and ammonia. The methane yield reported by Mshandete et al. (2004) for fish waste was 390 m^3CH_4/ton VS, lower than the theoretical yield of proteinous substrate (Moller et al., 2004); and they suggested that the ratio of inoculum to substrate (1:1) was the reason for the low yield. The high methane yield in the present study indicates that the BMP assay was probably performed under non-inhibited conditions.

The characteristic pungent smell that can emanate from fish waste was completely absent after anaerobic digestion. The pungent smell of fish waste has been attributed to the oxidation of unsaturated fatty acids (Rai et al., 2010) and protein degradation leading to the formation of hydrogen sulphide and ammonia respectively. Oil and fish protein hydrolysate extraction and separation (Mbatia et al., 2010b) followed by a further anaerobic biodegradation step could be expected to greatly reduce the smell.

3.2.2 Co-digestion of fish sludge and Jerusalem artichoke

Co-digestion of different kinds of waste may lead to either synergism or antagonism. Synergism is occurs when an additional substrate contributes essential nutrients needed for bacterial growth, or dilutes the toxic effect of already present compounds. In antagonism, the toxic effect of a compound is further exacerbated by the addition of another compound. The experimental yield for the 1:1 mixture of fish sludge and Jerusalem artichoke was 531 ± 10 m^3CH_4/ton VS added, compared with 402 ± 3 m^3CH_4/ton VS added for the 1:3 ratio fish sludge and Jerusalem artichoke mixture The experimental and calculated methane yields demonstrated in Figure 2 for the mixtures of fish sludge and Jerusalem artichoke were in good agreement for each mixture. The co-digestion methane yields were higher than mono-digestion of Jerusalem artichoke but lower than in mono-digestion of fish sludge. It was also observed that the methane yield increased with increased portion of fish sludge in the co-digestion assays. This finding indicates that no inhibition occurred in both mono and co-digestion BMP assays probably due to the large excess of nutrient rich inoculum as compared to the feedstock.

The average CH_4 contents of the biogas produced from the co-digestion of 1:1 and 1:3 mixtures were 70% and 67%, respectively. These values are higher than those normally obtained from conventional anaerobic digestion of organic waste conducted in single-stage slurry digesters (Samani, 2001). This is due to the high methane content of the biogas from the fish residues, 75-80%, which is higher than the typical methane concentration in a carbohydrate-dominated waste such as Jerusalem artichoke (50-60%).

3.3 Limitations of fish residues as a substrate for biogas production

The potential problems of using fish waste/fish sludge as a substrate in biogas production can not be fully demonstrated in a BPM assay, where the substrate is mixed with a high amount of inoculum. The chemical analysis of the waste in combination with the interpretation of the results from the BMP assay will, however, allow the analysis of the problems that might occur.

3.3.1 Inhibition by ammonia

Ammonia is produced through the biological degradation of protein-rich material. Ammonium, NH_4^+, and free ammonia NH_3, are the principal forms of inorganic ammoniacal nitrogen in aqueous solution. NH_3 has been suggested to be the main cause of inhibition since it can permeate cell membranes (Chen et al., 2008). This hydrophobic molecule may diffuse passively into the cell, causing proton imbalance and/or potassium deficiency (Chen et al., 2008).

The inoculum had an NH_4-N concentration of 4.0 g/L at a pH of 8. Rapid methane production from the control substrate in the BMP assay (the crystalline cellulose) indicated that the inoculum was well adapted for

degradation under high concentration of NH_3 (results not shown). The fish sludge had a total nitrogen concentration of 26.1 g/kg (Table 1), but the measured concentration of NH_4-N after the BMP assay was only 4.2 g/kg, since the inoculum was added to the fish sludge at high excess. The NH_3 at completion of the BMP assay has been calculated to be 532 mg/L. This is a relatively high value, indicating that NH_3 may be inhibitory, especially in if the system not acclimated. An NH_3 level of 150 mg/l has been observed to cause growth inhibition in non-acclimated systems, while an acclimated system can withstand up to 1100 mg/L NH_3 levels; the adaptation being attributed to a shift in the microbial degradation pathway (Hansen et al., 1998; Schnurer and Nordberg, 2008). In designing and operating an AD process, a substrate containing such high amounts of nitrogen must be added with care so as not to cause inhibition by free ammonia.

3.3.2 Light metals

The fish sludge abounds in light metals such as sodium, potassium and calcium, with calcium concentrations as high as 13 g/L (Table 1). Generally, high salt concentrations may dehydrate bacterial cells due to osmotic pressure, although microbes in a saline environment have been reported to accumulate or synthesize substances (osmolytes) which may aid water retention by the cells. Although moderate levels of sodium (100-200 mg/L) are beneficiary to anaerobes, for example, in the formation of ATP and the oxidation of NADH, high levels can be detrimental. High levels of potassium, for example, may facilitate the passive influx of K^+, thereby neutralizing membrane potential (Chen et al., 2008). This may have negative effects on the membrane integrity, thereby jeopardizing transport, as well as protective and nutritional functionalities. The high concentration of calcium in fish sludge means that carbonate and phosphate may be

precipitated, leading to loss in buffering capacity, scaling of reactors pipes, and the scaling of biomass, hence reducing specific activity.

It is worth mentioning that NH_3 has been reported to be antagonistic to light metal ions such as Na^+, Ca^{2+} and Mg^{2+} inhibition; a condition where the presence of one toxicant cancels out the toxic effect of the other and vice versa (Chen et al., 2008). In the same study (a review), Chen et al. (2008) discussed the mutual antagonistic effect of NH_3 and Na^+, also the presence of both Na^+ and K^+ or Na^+ and Mg^{2+} was reported to lead to an increase in methane production as oppose to a scenario where there was only Na^+. From this literature review and based on the chemical composition of fish sludge, it is plausible to think that inhibition will be less severe in the anaerobic digestion of fish sludge compared to a scenario where there were no metal ions e.g. in the anaerobic digestion protein rich substrate such as manure (Hansen et al., 1998).

3.3.3 LCFA inhibition

Fish waste is rich in oils (Figure 1). The lipid concentration in the fish sludge was not analysed, but the presence of high amounts of lipids is evident from the high methane yield (Figure 2). Lipid degradation products, LCFAs, have been reported to severely inhibit methanogenesis (Cirne et al., 2007; Luostarinen et al., 2009). This, albeit reversible, inhibition, is mainly due to the adsorption of LCFAs to bacterial biomass, causing floatation and precipitation. This creates a physical barrier impeding the transfer of substrates and products, leading to a delay in the initial methane production (Pereira et al., 2005). Substrates containing high amounts of lipids are therefore attractive due to their high methane yields, but they must be mixed with other types of substrates.

3.4 Features of a biogas process using fish sludge

To illustrate that a feasible biogas process can be designed to fully utilize fish sludge, calculations were made based on the laboratory-scale results. The calculations were based on the annual processing of 1000 tons of fish waste, providing 604 tons of fish sludge after extraction of the oils and FPH (Table 1). Mixing the fish sludge with Jerusalem artichoke residue at a ratio of 1:3 based on the VS content means that 2620 tons of the leaves and stems of Jerusalem artichoke must be added per year (Table 2). The process simulated was a one-stage continuously stirred tank reactor, which when ideally mixed gives concentrations in the reactor equal to the concentrations in the outflow. The organic loading rate was set to 3 kg VS/m^3 d, which is normal for a co-digestion process, and the TS in the reactor was set to a maximum of 8% as an increase in viscosity has been observed at and above a reactor TS of 9%, making proper mixing of the reactor contents difficult (FNR, 2010). The experimentally obtained input data and the calculated outputs are summarized in Table 2.

In Scenario A only the above mentioned substrates were added, while in Scenario B water was added to the substrates. The effluent concentration in Scenario A, which also represents the concentrations in the reactor of both TS and NH_4-N, will be too high, 12.5% and 8.6 g/L, respectively. In Scenario B, water was added to achieve a limit of 8% TS in the reactor. This causes the dilution of NH_4-N to 5.5 g/L. This is within the range where the microbial consortia will be influenced by NH_3, but the process is likely to be feasible (Schnurer and Nordberg, 2008). Adding water while keeping the reactor volume constant will decrease the hydraulic retention time, but the resulting 53 days for Scenario B is satisfactory and likely to

give a high, stable methane production in combination with a high NH_3 concentration (Schnurer and Nordberg, 2008).

Table 2 Experimental input and calculated output for biogas production from fish waste

Input data		
	Fish sludge	Jerusalem artichoke
Amount (ton/year)	604	2 620
Amount (ton VS/year)	190	570
Methane yield (Nm^3/ton VS)	742	283
Methane content in biogas (%)	79	55
Outputs		
Scenario	**A**	**B**
Methane production (MWh/year)	3007	3007
Methane content in biogas (%)	65	65
Active reactor volume (m^3)	690	690
Hydraulic retention time (d)	78	53
Effluent (bio-fertilizer)		
Amount (ton/year)	2 690	4 210
TS (%)	12.5	8.0
Ammoniacal nitrogen (g/L)	8.6	5.5
Process water addition (ton/year)	-	1 520

Table 2 also shows the total input and output in a co-digestion process for the biodegradation of fish sludge under non-inhibited conditions. Considering a calculated methane content of 65%, 538 tons or 466 000 m^3, of biogas can be produce produced per year. This has and an energy content of 3 GWh, approximately equivalent to the energy contained in 300 m^3 of diesel fuel.

The heavy-metal content of the bio-fertilizer meets the Swedish requirements on the use of effluent as a bio-fertilizer (SP, 2010). Table 3 shows the nutrients, heavy metals and guidelines values as recommended

by SPCR 120. The contents of NH_4-N, P and K are in line with, or higher than those of e.g. swine or cattle manure, which should make this effluent an attractive bio-fertilizer. The heavy metals, cadmium (Cd), lead (Pb), zinc (Zn), nickel (Ni), chromium (Cr), copper (Cu) and mercury (Hg) were also within the guidelines limit values. However, waste of animal origin must also meet the demands on hygienic quality, which can be achieved by treating the waste at 70°C for 1 hour. Alternative treatment with proven similar effects on pathogen reduction can also be approved, and 55°C for 10 hours is one suggested alternative. During oil extraction (Mbatia et al., 2010b) the fish waste was pre-treated at 55°C for 1 hour, and prolonging the holding time did not affect the oil yield. The holding time could thus be extended to 10 hours in this extraction step, to ensure that the hygienic quality standard is met, and no separate hygienization would then be needed. The bio-fertilizer produced in the suggested process is deemed to be an attractive high-quality product.

Table 3 Characteristics of the effluent (bio-fertilizer) for a simulated full-scale anaerobic digestion process. Values given in brackets are the maximum concentrations for the certification of biogas plant effluent as a bio-fertilizer in Sweden (SP, 2010)

Parameters	**Bio-fertilizer scenario A**
TS (%)	8.0
VS (%)	5.2
Concentrations (mg/kg wet weight)	
Ntot	7100
NH4-N	5500
P	1500
S	450
K	1100
Na	290
Ca	5710
Mg	980
Cd	0.06 (0.08)
Pb	0.35 (8.0)
Zn	13.2 (64)
Ni	0.17 (4.0)
Cr	0.13 (8.0)
Cu	0.90 (48)
Hg	0.01 (0.08)

4. Conclusions

The results of this study demonstrate that the different components of fish waste can be converted into several useful products. Through anaerobic digestion, fish sludge can be converted from an odorous residue to a renewable energy carrier and a high-quality bio-fertilizer. However, fish sludge cannot be digested alone due to its high content of potentially inhibitory compounds. Co-digestion, exemplified in this study by a residue

from crop production, could mitigate the potential inhibitory effect of NH_3, light metals and LCFAs, as these inhibitors are degraded or diluted to acceptable levels.

5. Acknowledgements

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Paper VI

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Effects of anaerobic pre-treatment on the degradation of dewatered-sewage sludge

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ABSTRACT

Effects of anaerobic pre-treatment were evaluated on the dewatered-sewage sludge from a municipal wastewater treatment plant in order to improve its biodegradability through anaerobic digestion. The pre-treatment was conducted in laboratory scale at 25, 50 and 70 °C for an incubation time of two days. As a reference, sludge sample was also autoclaved at 121 °C for 20 min to determine the thermal effect to the subsequent sludge digestion. Characteristics of dewatered-sludge such as viscosity, pH and soluble chemical oxygen demand (SCOD) were affected by the pre-treatment. A higher SCOD after the pre-treatment did not necessarily imply an increase in methane yield, although initial biodegradability rate was improved. In fact, a 'great' improvement in SCOD concentration (up to 27%) was translated in only 8% increase in the methane yield (298 ± 9 and 276 ± 6 Nml CH_4 gVS^{-1}_{added} for pre-treated and untreated samples, respectively). Increasing the anaerobic pre-treatment time from 12 h to 2 days at 50 °C led to an 11% improvement in methane yield. Methane content in biogas increased from an average of 65–69% for the pre-treated and untreated substrates, respectively. Volatile solids (VS) reduction increased from 42% to 51%. The overall digestion time was not affected by the pre-treatment but 90% of methane was produced in the first 12 days of incubation for 50 °C pre-treated samples whereas it took 2–5 days more for 25, 70 °C pre-treated and untreated sludge samples. In this study, thermophilic digestion was also found to be a better option in terms of faster digestion and higher VS-reduction, but it showed lower methane yield as compared to mesophilic digestion, i.e. 9% and 11% increment in methane yields for thermophilic and mesophilic digestions, respectively.

1. Introduction

Anaerobic digestion (AD) is a technology that can extract methane-rich biogas from biological degradation of regionally abound biomass such as municipal solid wastes and wastewaters. The process is a multi-step microbiological degradation process comprising hydrolysis, acidogenesis, acetogenesis and methanogesis. Hydrolysis is known to be the rate-limiting step especially in the degradation of complex substrates containing particulates such as sewage sludge from wastewater treatment plants [1]. The efficiency of anaerobic degradation may be improved by incorporating a pre-treatment step that will enhance the hydrolysis of particulate organic matter. Pavlostathis and Gossett [2] stated that the rigid structure of the microbial walls, which prevent the inner cell products from leaking out hampered digestion of sewage sludge. Sludge solubilisation is therefore a logical approach to improve digestion of sewage sludge. Several methods have been investigated to hydrolyse or solubilise sludge. These include mechanical pre-treatment which is good in solubilising microbial cells but complicated and expensive [3]. Chemical and thermal treatments are based on strong acidic or basic conditions in combination with high temperatures and pressure [1]. Chemo-thermal pre-treatment is efficient in enhancing sludge digestion; however the aggressive reaction conditions often impose special material requirements [4]. Thermal pre-treatment is reported to be efficient in sludge hydrolysis but it consumes a substantial amount of energy and in some cases, there is formation of toxic, refractory compounds during pre-treatment which is a major drawback [5] and deactivation of enzymes may occur. Mullar [6] explained the formation of the refractory compounds as being a result of Maillard reaction. In this reaction, sugars and amino acid react to form melanoids which are known to be inhibitory to the methanogens down stream. The formation of hardly degradable materials (e.g. the possibility of the formation of dioxins at temperatures of 200 °C) has also been reported [7]. High temperature pre-treatment of sewage sludge though may greatly reduce pathogen concentrations in sludge is expensive, difficult to operate and does not significantly improve methane yield [8,9]. As an alternative

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approach, biological pre-treatment has the potential to be more cost efficient, this is because endogenous enzymes secreted by bacteria present in the sludge can enhance hydrolysis. The purpose of this study was to investigate the effects of anaerobic pre-treatment at moderate temperatures (from 25 to 70 °C) and holding time (from 12 h to 3 days) on anaerobic degradation of dewatered-sewage sludge. Previous studies have been carried out on thermal pre-treatment at 70 °C [10]. In the present study, emphasis was laid on not only the effects of thermal pre-treatment but most importantly on the biological effects. Base on our literature search, no study has been reported on anaerobic pre-treatment of sewage sludge to improve biodegradability.

2. Experimental

2.1. Sample and inoculums

The dewatered-sewage sludge was collected from Källby WWTP in Lund, Sweden. This sludge was obtained by collecting the primary, secondary and tertiary treatment sludge and thickened by a dewatering process with the aid of a high molecular flocculants based on polyacrylamide. The sludge used in the present study has a ratio of 1:3 between the secondary sludge and the primary sludge. The total solids (TS) of dewatered-sewage sludge ranged from 8 to 9% (w/w) and volatile solids (VS) was about 75% of TS. Thermophilic seed sludge collected from Källby anaerobic digester (Sweden) had TS of 5.61% (w/w) and VS was 56% of TS. The mesophilic seed sludge was collected from the anaerobic digester in Ellinge WWTP (Eslöv, Sweden). TS was 4.31% (w/w) and VS was 47% of TS. Fresh sludges were collected for each experiment and stored at 4 °C prior to use. Characteristics of dewatered-sewage sludge and inoculums are listed in Tables 1 and 2.

2.2. Analytical methods

The TS, VS and pH were determined before and after digestion according to standard methods [11] to evaluate the physico-chemical changes in sludge after the pre-treatment and the methane potential test. Biogas composition at the headspace was determined using a gas chromatograph, thermal conductivity detector (Agilent Technologies 6890N CA, USA) equipped with a Haysep N 80/100 mesh and molecular sieve columns separated with valves. Helium was the carrier gas with a flow rate of 28 ml min^{-1} Temperatures of detector, injector and column were 150, 105 and 60 °C, respectively. The compounds detected were methane, carbon dioxide, oxygen, and nitrogen. Total gas volume was measured using a graduated 100-ml gas-tight syringe with a sample lock (Fortuna, Germany). Methane yield was calculated as the net amount of methane produced per unit VS added to the digester and corrected to standard pressure (760 mm Hg) and temperature (0 °C) and zero water vapour. Sample weight was determined with the aid of a 3-digit precision scale balance. VFAs were analysed with HPLC, Varian Star 9000 (Varian, Walnut, USA), with a Biorad column, Cat. 125-0115 (Hercules, USA) as was previously described by Parawira et al. [12]. The alkalinity was evaluated as partial alkalinity (PA) by titrating an aliquot of 4000 g centrifuged sample to a 5.75 end-point and total alkalinity (TA) by titration to a 4.3 end-point with the aid of TIM800 Titration Manager (Radiometer, Copenhagen, Denmark). The SCOD was determined by oxidation of the organic compounds with $K_2Cr_2O_7$. The Cr^{3+} produced thus was analysed calorimetrically as previously described by Tiehm et al. [13]. The degree of COD solubilisation was calculated by the following formula:

$$\text{COD solubilisation } (\%) = \frac{\text{Soluble COD (SCOD) measured after pretreatment}}{\text{Total COD (TCOD) measured before pretreatment}} \times 100$$

Table 1
Dewatered-sludge characteristics before and after pre-treatment (TS and VS values reported are averages of two measurements).

Variables	Inoculum	Untreated	70 °C P.T.	50 °C P.T.	25 °C P.T.
TS (%, w/w)	5.61	9.03	8.89	9.01	9.01
VS (%, TS)	55.25	66.78	65.35	64.92	66.92
CO_2 (ml/flask)	0	0	7.1	6.2	9.3
CH_4 (ml/flask)	0	0	2.4	1.5	3
pH	8.3	6.8	6.6	6.4	5.8
TCOD (g/l)	NA	92.00	–	–	–
SCOD (g/l)	NA	2.12	19.95	20.65	10.71
COD solubilisation (%)	–	2.0	21.7	22.5	11.6

2.3. Anaerobic pre-treatment

The basic experimental set-up for pre-treatment was a 500 ml Erlenmeyer flask into which 300 ml of dewatered-sewage sludge was introduced, flushed with nitrogen gas for 3 min in order to create anaerobic conditions. The flasks were immediately corked with butyl rubber septa. The flasks were incubated at room temperature (25 ± 2), 50 and 70 °C in shaking water baths (GFL 1086; Gesellschaft fur Labortechnik GmbH, Burgwedel, Germany) at a frequency of 70 rpm, for 12 h, 1 day, 2 days and 3 days. For a second trial, pre-treatment was done only at 50 °C and the treatment time varied from 12 h to three days. During the pre-treatment, CO_2 and CH_4 gases were produced and quantified in ml/flask. Samples were also autoclaved at 121 °C for 20 min to study solely the thermal effect of pre-treatment on the subsequent sludge digestion. All tests were carried out in triplicates. Statistical analyses were performed using Microsoft excel 2003 program, and the data of recurrent experiments were given as the mean ± standard deviation (SD).

2.4. Methane potential test

The pre-treated sludge and untreated (control) sludge were used as substrates for the methane potential test to assess sludge biodegradability at both mesophilic and thermophilic conditions. The inoculum to substrate ratio (ISR) was set at 1:1 gVS. The basic experimental unit consisted of a 500 ml Erlenmeyer flasks incubated at 37 °C in shaking water baths (GFL 1086; Gesellschaft fur Labortechnik GmbH, Burgwedel, Germany) at a frequency of 70 rpm. The active volume was 300 ml, anaerobic conditions were established by flushing the headspace of flasks with nitrogen for 3 min, and the flasks were immediately sealed with butyl rubber septa. An outlet in the stopper was used for biogas collection in a gas-tight aluminium foil bag for each experimental unit. All tests were conducted in triplicates. During the experiment, gas composition and total gas volume were monitored on a daily basis. VFAs, pH, TS, VS and alkalinity were determined at the end of each batch experiment. Thermophilic and mesophilic anaerobic assays were

Table 2
Characteristics of dewatered-sludge after 50 °C anaerobic pre-treatment (TS and VS values reported are averages of two measurements).

Variables	Inoculum	Untreated	12 h	1 day	2 days	3 days	Autoclave
pH	7.61	6.40	6.07	6.16	6.08	6.12	6.30
TS (%, w/w)	47.10	74.33	82.71	74.41	67.94	73.07	77.31
VS (%, TS)	2.03	6.40	6.70	6.40	5.30	5.70	6.51
SCOD (g/l)	NA	1.93	13.02	14.31	16.11	20.62	24.49
TCOD (g/l)	NA	89.10	–	–	–	–	–
COD solubilisation (%)	–	2.0	14.6	16.1	18.1	23.2	27.2
CO_2 (ml)	NA	NA	170	180	258	427	NA
CH_4 (ml)	NA	NA	5.5	6.0	5.4	11.7	NA

conducted using thermophilic inoculum to evaluate the effect of operational temperature on degradation of dewatered-sewage sludge. This was done to simulate the 'state of affairs' of the biogas plant at the wastewater treatment plant (Källby, Sweden), where the mesophilic (37–40 °C) operation is carried out in winter and thermophilic (50–55 °C) operation is performed during the rest of the year. Controls containing only the inoculum were used to measure the indigenous methane production from the inoculum and this was subtracted from the total methane production. The experiments were run for about a month and terminated when methane production was less than 5 ml day^{-1}. Methane yields were normalised by correcting the temperature to 0 °C and pressure to 760 mm Hg.

3. Results and discussions

3.1. Anaerobic pre-treatment

Both biological (anaerobic) pre-treatment and thermal treatment led to changes of the physico-chemical characteristics of sludge. For instance, pH decreased up to 1 pH unit (Tables 1 and 2). The decrease in pH can be explained by the formation of acidic compounds through the depolymerisation of the organic matter by enzymes produced by micro-organisms present in mainly the secondary sludge [14,15] and or lysis of the cell through heat action [16]. It seems that organic compounds such as lipids, carbohydrates and proteins were degraded in order to form volatile fatty acids and amino acids (soluble monomers) during the pre-treatment, which decreased the pH [17]. Particulate polymeric organic compounds were therefore transformed into soluble monomeric compounds. Studies have showed that these monomeric compounds produced during chemical or biological sludge pre-treatments consist mainly of VFAs [18]. These soluble monomers were transferred into the liquid phase thereby increasing the SCOD. At ambient temperature, COD solubilisation of about 2% (SCOD of 1.9 g l^{-1}) was obtained. The 2% SCOD at ambient temperature was comparable with the 3.1% obtained by Yuko et al. [19] during the solubilisation of excess activated sludge by self-digestion. In the present experiment, the substrate was mainly primary sludge which is easily fermented. Autoclaved and 50 °C anaerobic pre-treated samples gave the best pre-treatment results with COD solubilisation of 27.15 and 23.13% respectively (Tables 1 and 2). Previous studies by Kim et al. [20] on waste activated (secondary) sludge had shown 36.7% for thermal pre-treatment and 64.4% for thermo-chemical pre-treatment. Bougrier et al. [21] termed this phenomenon as solubilisation which represents the transfer (of COD or solids) from the particulate fraction of the sludge to the soluble fraction of the sludge. It should be noted that these VFAs are the main precursors of methane formation. Studies have shown that 70% of methane in an anaerobic system is produced from VFAs in the form of acetic acid [22].

Li and Noike [4] reported that the optimal pre-treatment temperature and incubation time for improving AD of sludge were 170 °C and 60 min, however in the current study, autoclaving which was done as a reference to the biological pre-treatment was carried out for 20 min at 121 °C. SCOD improvement was considered as a result of break down of sludge cells (de-agglomeration and disintegration of flocs) and the content released into the digestion broth.

Samples pre-treated at room temperature (25 °C) showed the lowest pH of 5.8 as compared to 6.6 and 6.4 for 70 and 50 °C pre-treated sludge (Table 1), this is most likely due to the higher concentration of carbon dioxide (acidic gas) in the liquid phase at lower temperatures [23].

The solubilisation of insoluble biomass and increase of soluble biomass in the pre-treated sludge samples could be due to both biological and thermal effects during anaerobic incubation at various temperatures. Pre-treatment at room temperature has mainly biological effect whereas both biological and thermal effects were involved for 50 °C and 70 °C anaerobic pre-treatment. Jacob [24] reported that anaerobes that hydrolyse sludge (through the intermediate of secreted hydrolytic enzymes) were quite active within the temperature range from 60 °C up to 75 °C. Similarly, Li and Noike [4] reported that improvement in hydrolysis could be observed at temperatures above 60 °C. This enhanced enzymatic action could be the reason for the increased sludge solubilisation at higher incubation temperatures than at the room temperature. The viscosity of the dewatered-sewage sludge was reduced especially with thermal pre-treatment (visual observation). The original viscous semi-solid sludge mass was transformed into a more fluid sludge mass after the pre-treatment. This can be likened to the 'potato effect' reported by Hans [23]. In his report, the action of thermal hydrolysis of dewatered-sludge was described as being similar to cooking a potato, resulting in volatile solids being easily digested. Solubilisation as a result of death of the bacteria cells was considered minimal in the current study as a very small fraction of

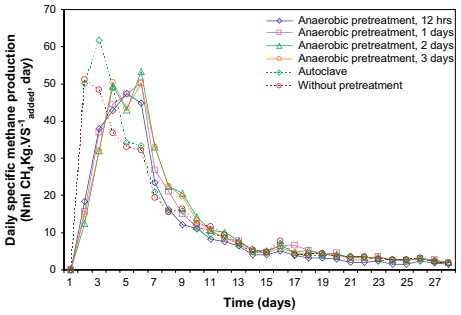

Fig. 1. Typical daily methane production curve in terms of Nml CH_4 gVS^{-1}_{added} for the degradation of non-pre-treated and pre-treated dewatered-sewage sludge. (Nml CH_4 = normalised ml CH_4 at 0 °C and 1 atm).

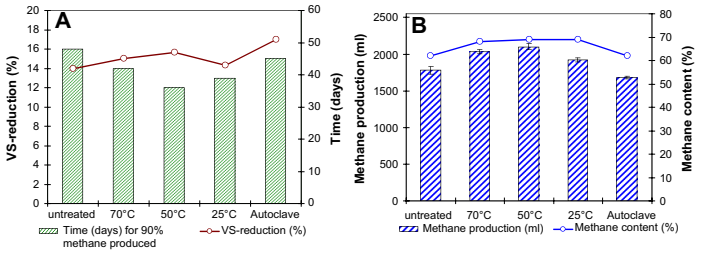

Fig. 2. Total methane production, methane content and VS-reduction during anaerobic pre-treatment of dewatered-sewage sludge.

the substrate was secondary sludge. Decreases in TS and VS during the pre-treatment could be due to the conversion of some carbonaceous solids to carbon dioxide and methane during pre-treatment (Tables 1 and 2).

3.2. Methane potential tests

3.2.1. Anaerobic digestion of 25, 50, 70 °C pre-treated sludge samples

Methane potential tests were conducted in order to determine the effect of anaerobic pre-treatment for subsequent biogas (methane) production. The methane from the inoculum was withdrawn and α-cellulose powder (Sigma) with a methane yield of 399 ± 12 Nml CH_4 gVS^{-1}_{added} was obtained as a reference substrate to indicate the microbial activity of the inoculum. This value is close to theoretical 415 Nml CH_4 gVS^{-1}_{added} for carbohydrate reported by Åsa [25]. Methane potential results are shown in Figs. 1–3. Methane production rate for pre-treated sludge samples was higher compared to the untreated samples (Fig. 2B). However, in all treatments little differences in methane yields were observed (Fig. 3). It should also be noted that most of the methane was produced during the early phase of degradation (Fig. 1). In fact 90% of methane was produced within the first two weeks of fermentation for both pre-treated sludge and non-pre-treated sludge. Pre-treated samples at 50 °C shown a particular shorter time of 12 days for 90% methane produced (Figs. 1 and 2A). Daily peak methane production also occurred within the first week of digestion. Non-pre-treated sludge showed daily peak methane production in the 2nd day of digestion while pre-treated sludge showed peak methane production from the 4th to the 6th day of digestion. This could be because the existing easily degradable COD was consumed during the pre-treatment phase, while the delayed prolonged peak methane production could be due to the increased SCOD as a result of pre-treatment. There were therefore more easily degraded organic compounds for the pre-treated sludge samples than the untreated sludge samples. The trend of the degradation curves from the 3rd day for non-pre-treated sludge sample and from the 4th and 6th day for the pre-treated sludge samples is as results of the degradation of both SCOD not easily degraded particulate COD. The trend from the 15th day was probably the degradation of particulate COD (Fig. 1). The total methane production resulted from both dissolved and particulate COD. In the case of soluble readily biodegradable COD, micro-organisms take up the substrate and metabolised it whereas for particulate slowly degradable COD, the substrate must be sorbed onto the micro-organisms and broken down to simple chemical units by external enzymes before taken up and metabolised [26].

The highest normalised methane yields in order of 293 ± 4 and 298 ± 4 Nml CH_4 gVS^{-1}_{added} were observed for 50 °C and autoclave pre-treated samples respectively. This indicated a 7 and 8% increment in methane yield for 50 °C and autoclave pre-treatments, respectively (Fig. 2 and Table 3). The rest of the pre-treated samples (70 and 25 °C) were a little higher or in the same range as the untreated samples. Data of methane yields obtained in this study are comparable to those reported by Bougrier et al. [21] for the ultrasonic, thermal and ozone pre-treatments of sewage sludge.

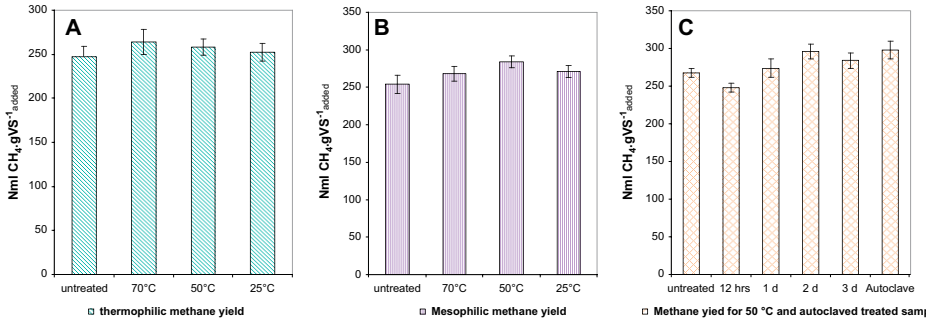

Fig. 3. (A) Normalised methane yields (Nml CH_4 gVS^{-1}_{added}) after thermophilic; (B) mesophilic anaerobic digestion of pre-treated sludge; (C) methane yield for 50 °C pre-treated sample.

Table 3
Methane yield (MY Nml CH_4 $gVS_{added})^{-1}$, methane content (MC%), VS-reduction (VR%), partial alkalinity (PA mg l^{-1}), total alkalinity (TA mg l^{-1}) VFAs (mg l^{-1}) and pH after anaerobic digestion of pre-treated dewatered-sewage sludge.

Test	MY	MC	VR	PA	TA	VFAs	pH
70 °C	280 ± 6	68	45	5172	6499	454	7.51
50 °C	293 ± 9	69	46	5340	6648	203	7.52
25 °C	279 ± 5	69	42	5382	6558	212	7.56
Autoclave	298 ± 9	64	51	5357	6748	157	7.50
Untreated	276 ± 7	62	42	5211	6266	200	7.60

This implies that pre-treatment enhanced the early degradation steps of AD (hydrolysis and acidogenesis) as can be seen from the improvement of SCOD (Table 2), but had a lesser effect on the down stream steps (acetogenesis and methanogenesis). The increase in VS-reduction from 42 to 51% had no correlation with the overall degradation speed and methane yield. A higher VS-reduction did not immediately lead to higher methane yield (Tables 3 and 4). This indicated that organic compounds were not all converted to biogas but probably to some other compounds. Organic compounds, both soluble and particulate could have been used by micro-organisms for growth, repairs and multiplication. This may also explain why an increase in solubilisation does not always translate into increase methane production. However, the VS-reductions in the present study were in the same the range as the 40–60% reduction reported by Parkin and Owen [27] for the anaerobic digestion of primary sludge.

3.2.2. Anaerobic digestion of 50 °C pre-treated sludge sample

Samples pre-treatment carried out at 50 °C resulted in a higher methane yield. This was further tested in a separate experiment by varying the pre-treatment time. Samples pre-treated for 2 days showed better methane yields. Improvement of methane yield was in the range of 11% both for samples pre-treated at 50 °C for two days (Fig. 3C). However, pre-treated samples with incubation time of three days and autoclaved samples resulted in higher solubilisation (20.62 and 24.49 g SCOD L^{-1}) (Table 2). The pre-treatment led to solubilisation but not all solubilised substrate was converted into biogas. The results showed that a high degree of solubilisation from a pre-treatment does not necessarily lead to an increase in methane yield. Formation of recalcitrant and inhibitory compounds to methanogens during pre-treatment such as dioxin and melanoids has been reported with thermal pre-treated sludge samples [6]. Muller [28] also reported that these melanoids could start forming at temperatures less than 100 °C. This could explain the lower methane yield for 70 °C pre-treated sludge samples as to compare to 50 °C pre-treated samples.

3.2.3. Thermophilic vs mesophilic digestion of 25, 50 and 70 °C pre-treated sludge samples

The degradation rate under thermophilic condition was much faster than the digestion rate carried out under mesophilic condition (Fig. 4). It took approximately two weeks for 90% of methane to be generated under thermophilic conditions while it was almost a month for mesophilic digestion. This could be due to higher specific growth rate of thermophilic microbes compared to their mesophilic analogues [29]. De la Rubia et al. [30] reported that hydrolysis was faster step under thermophilic condition than under mesophilic conditions, while it is the opposite as far as the methanogesis is concerned. The methane content in biogas and methane yield were higher for the mesophilic digested sample than for the thermophilic digested samples (Table 3 and Fig. 3A and B). This could be due to the decrease in CO_2 solubility caused by raised operational temperature. There was 11% improvement in methane yield for digestion of pre-treated sludge samples at mesophilic conditions as opposed to a 9% increment for digestion at thermophilic conditions. Methane production under mesophilic condition with thermophilic inoculum increased dramatically in the second week (Fig. 4). It seems the micro-organisms took approximately two weeks to get acclimatised to the mesophilic condition. The relatively fast adaptation of micro-organisms to mesophilic conditions indicates the presence of mesophilic micro-organisms in the thermophilic inoculum. Chen [31] showed that there exist 9% of thermophiles and 1% obligate thermophiles in mesophilic sludge. In fact, the digester (Källby WWTP, Sweden) where the inoculum was collected operates in thermophilic (50–55 °C) condition in summer and in mesophilic condition (40 °C) in winter for energy saving.

3.2.4. General aspects of methane potential test

For all experiments carried out during this study, the methane content oscillated between 62 and 69% (Table 3). High methane content for pre-treated sludge samples could be as a result of increase in specific activity of methanogens [12]. Similar phenomenon has also been observed in the anaerobic digestion of thermal sludge pre-treated sludge samples [32]. It took about 30 days in all experiments to reduce daily methane production to less than 5 ml methane per flask. This indicated that pre-treatment had no effect on the total degradation rate but could only enhance the early degradation steps (i.e. hydrolysis and acidification).

Methane content and methane production were closely related (Fig. 2B). Samples pre-treated at 50 °C had the highest methane production and methane content. This could be as a result of a good combination of the biological (anaerobic) and thermal effects of the pre-treatment on the sludge degradation at this temperature.

The total volatile fatty acids ranged from 200 to 450 mg L^{-1} (Table 3) and the dominating VFAs were acetic and propionic acids at the end of the experiment. The initial pH ranged from 5.8 to 6.8 while the final pH values range from 7.5 to 8.2. The higher pH for thermophilic fermentation as compared to mesophilic fermentation (Table 4) is most likely related to the higher Henry's constant for carbon dioxide and a lower liquid concentration with increasing temperature [33]. Carbon dioxide being an acidic gas, a lower liquid concentration will lead to a higher pH value at the same alkalinity concentration [23]. The higher pH at thermophilic conditions can also be associated with a higher protein degradation which entails production of ammonia and hence an increase in pH. Total and partial alkalinity values were within optimal operation range (Table 3). This indicated that the pH was conducive for biogas production. The above parameters are within the optimal range

Table 4
Methane yield, methane content, VS-reduction, and pH after thermophilic and mesophilic anaerobic digestion of pre-treated dewatered-sewage sludge.

Variable	Thermophilic AD				Mesophilic AD			
	70 °C	50 °C	25 °C	Control	70 °C	50 °C	25 °C	Control
pH	8.2	8.2	8.1	8.2	7.9	7.9	8	8
VS-reduction (%)	41	42	39	38	42	42	39	39
Methane content (%)	68	68	66	63	69	68	67	64
Methane yield (Nml CH_4 gVS^{-1}_{added})	264 ± 8	268 ± 6	252 ± 7	247 ± 9	268 ± 7	284 ± 8	271 ± 6	254 ± 9

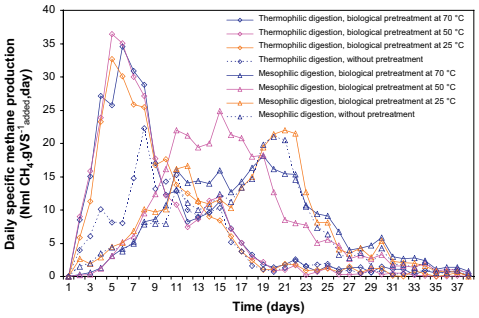

Fig. 4. Normalised methane yield (Nml CH_4 gVS^{-1}_{added}) rate during thermophilic and mesophilic anaerobic digestion (AD) of anaerobic pre-treated dewatered-sewage sludge.

reported by [34]. The optimal operating values were suggested as 6.8–7.2 for pH, 1500–3000 mg L^{-1} for alkalinity and 50–500 mg L^{-1} for VFAs in form of acetic acids. Marginal working values reported for pH were between 6.6 and 7.2, between 3000 and 5000 mg L^{-1} for alkalinity, and 500 and 2000 mg L^{-1} for volatile fatty acids.

4. Conclusion

Results from our experiments have clearly demonstrated that biological (anaerobic) or/and thermal pre-treatments could improve COD solubilisation by up to 27% as a result of the formation of simple monomeric compounds such as VFAs, amino acids and simple sugars. Improvement in methane yield as a result of the pre-treatment was in the order of 11% and VS-reduction was improved from 42 to 51%. Methane content in biogas was also increased from 62 to 60%. These results indicated that the impact of hydrolysis which is the rate-limiting step was reduced but did not lead to same measure in methane production. A major conclusion drawn from this study is that 90% of total gas was produced within the first 14 days of digestion prompting therefore the possibility to digestion dewatered-sewage sludge at a retention time as short as two weeks while keeping the methanogens and thereby avoiding cell wash out.

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